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VARIABILITY OF *IN VITRO* AND PHENOLOGICAL BEHAVIOURS OF COCOA HYBRIDS BASED ON DISCRIMINANT ANALYSIS

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ABSTRACT

Cultivated cocoa species (*Theobroma cacao* L.) is originated from tropical rainforests of South and Central America. Its fermented and dried seeds constitute the raw material for the chocolate manufacture. In order to analyse the variability of the *in vitro* and phenological behaviours of 6 cocoa hybrids, the typological and discriminant classifications were performed. These six hybrids developed were : L120-A2, L126-A3, L231-A4, L232-A9, L233-A4 and L330-A9. Three culture media known as PCG1, PCG3 and PCG4, only differing in hormonal concentration, were used as support to sow staminodes and petals from these hybrids. SCA6 and C151-61 were used as controls. After 3 months, callogenesis and embryogenesis variables were scored on each genotype. The Principal Component (PCA), Hierarchical Cluster (HCA) and Factorial Discriminant Analyses (FDA) were used. For the PCA, the number of embryogenic explants, embryos number obtained per embryogenic explant and embryogenesis percentage, as well as flowering level, fructification level and leaves flush were found to be relevant. For the FDA, only the number of callogenic explants and leaves flush were relevant, indicating that the parameters relevance seems to depend on analytical method. Genotypes from cluster C1, namely L120-A2, L126-A3, L231-A4 and L330-A9, expressed the highest callogenesis and leaf flush values. The first 3 were half sibs, with IMC67 as a male common parent. The discriminant function Z1 = -29.123 + 0.201*Flush + 1.71*Ncal discriminated in the proportion of 96.20% of the clusters identified. The second discriminant function Z2 did not succeed in discriminating the clusters identified. Indeed, the P from Wilks' Lambda which is associated with it was not significant. The equation Z1 allows for prediction of the cluster of belonging of a new individual from its callogenesis and leaf flush values. The 4 aforementioned hybrids could be used to produce cocoa aroma, theobromin and cocoa butter from cell suspensions in bioreactors.

Key Words: Callogenesis, Côte d'Ivoire, leaf flush

RESUME

Le cacaoyer cultivé (*Theobroma cacao* L.) est originaire des forêts humides tropicales de l'Amérique Centrale et du Sud. Ses graines fermentées et séchées constituent la matière première pour la fabrication du chocolat. Pour analyser la variabilité des comportements *in vitro* et phénologiques de 6 hybrides de cacaoyer nouvellement crées, les classifications typologique et discriminante ont été réalisées. Les 6 hybrides crées étaient : L120-A2, L126-A3, L231-A4, L232-A9, L233-A4 et L330-A9. Trois milieux de culture codés PCG1, PCG3 et PCG4, se différenciant seulement par leur concentration hormonale, ont été ensemencés avec les staminodes et les pétales issus de ces hybrides. Les génotypes SCA6 et C151-61 ont servi de témoins. Au terme de 3 mois de culture, les variables de callogenèse et d'embryogenèse somatique ont été mesurées sur chaque génotype. L'Analyse en Composantes Principales (ACP), la Classification Ascendante Hiérarchique (CAH) et l'Analyse Factorielle Discriminante (AFD) ont été réalisées. Concernant l'ACP, le nombre d'explants embryogènes, le nombre d'embryons produits par explant embryogène et le pourcentage d'embryogenèse ainsi que le niveau de floraison,

le niveau de fructification et le rythme des poussées foliaires ont été révélés pertinents. Concernant l'AFD, seuls le nombre d'explants callogènes et le rythme des poussées foliaires ont été identifiées pertinents indiquant que la pertinence des variables semble dépendre de la méthode d'analyse. Les génotypes issus du groupe C1, notamment L120-A2, L126-A3, L231-A4 et L330-A9 ont exprimé à la fois les plus hautes valeurs de callogenèse et de poussées foliaires. Les trois premiers sont demi-frères de parent mâle commun l'IMC67. La fonction discriminante Z1 = -29.123 + 0.201*Flush + 1.71*Ncal a discriminé dans la proportion de 96.20 % les groupes identifiés. La seconde fonction discriminante Z2 n'a pas pu discriminer les groupes identifiés. En effet, la P issue du Lambda de Wilks qui lui est associée n'a pas été significative. L'équation Z1 permet la prédiction du groupe d'appartenance d'un nouvel individu à partir de ses valeurs de poussées foliaires et de callogenèse. En raison de leur haute aptitude à la callogenèse, les 4 hybrides susmentionnés pourraient être utilisés pour produire l'arome de cacao, la théobromine et le beurre de cacao à partir des suspensions cellulaires dans des bio-réacteurs.

Mots Clés: Callogenèse, Côte d'Ivoire, poussées foliaires

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a forest tree species, which provides raw materials for chocolate. Côte d'Ivoire is the biggest producer of cocoa, contributing about 44.25% of the world supply (ICCO, 2005). Over six millions people depend directly or indirectly on cocoa; and accounts for 30% of working population (Anonymous, 2004).

Average yield of dried cocoa ranges from 400 to 800 kg ha^{-1}, which is compared to 2.5 t ha^{-1} recorded on research stations (Mossu, 1990). One of the means to increase cocoa yields on farm is through the development of new varieties. Thus, in 1988, 28 hybrid developed progenies were planted in the old field of the CNRA Station, namely C2/3 located at Bingerville (N'goran, 1988). Out of thirty hybrid individuals preselected, six (L120-A2, L126-A3, L231-A4, L232-A9, L233-A4 and L330-A9) were tested *in vitro* for vegetative propagation via somatic embryogenesis technique. During the collection of flowers, the following 3 phenological variables were recorded, namely, flowering, fructification and leaf flush. It was demonstrated that phenological character variations depend mainly on endogenous plant growth regulators such as indole-3-acetic acid and gibberellins, among others (Kofler, 1969). In the same way, Alemanno (1996) was able to assay endogenous Indole-3-acetic (IAA) and abscisic acid (ABA) in 2 genotypes, especially embryogenic and non-embryogenic. Therefore, the endogenous plant growth regulators influence the callogenesis and somatic embryogenesis (Tan and Furtek, 2003; Issali *et al*., 2009).

Little is known about the structuring of some cocoa hybrids using simultaneously callogenesis, somatic embryogenesis and phenology parameters. The objective of this study was to structure the variability of the *in vitro* and phenological behaviours of 6 cocoa hybrids using a multivariate approach.

MATERIALS AND METHODS

Plant materials were constituted of 6 promising hybrids, preselected for yield and resistance to Phytophthora pod rot (Lachenaud *et al.*, 2001). These were L120-A2, L126-A3, L231-A4, L232-A9, L233-A4 and L330-A9. These hybrids were obtained from crosses between upper amazon parental clones, namely Pa13, Pa121, IMC67, Pa150, and P19A. The first 3 hybrids are half sibs with IMC67 as common male parent; whereas the last 3 are half sibs with Pa150 as common male parent.

Trees were planted in a completely randomised design, in the experimental field C2/1 of the ancient station of Centre National de Recherche Agronomique (CNRA), Côte d'Ivoire. Clones SCA6 and C151-61 were used as controls. The former was identified like very embryogenic (Maximova et *al*., 2002), while the latter descends from back crossing ICS1 x (ICS1 x SCA6). The former was planted in field B10, while the latter was it in field C2/1. All of these fields were located at Bingerville Research Station in Côte d'Ivoire.

Forty trees were planted per progeny. A border composed of 138 trees was associated with the design. Trees in a given row were sown at a spacing of 2.5 m, and inter-row spacing of 3 m.

This corresponds to density of 1333 trees ha^{-1}. The experiment was carried for 2 years.

The study was conducted at the ancient station of CNRA situated at Bingerville, located at 3°52'59" West and 5°21'42" North in Côte d'Ivoire. For 2 years of the study, the weekly pluviometric total, weekly average maximum temperature, weekly average minimum temperature, total sunshine and weekly average relative humidity were 4186.80 mm, 30.63°C, 20.16°C, 77651.42 hours, 82.13%, respectively.

Culture initiation and monitoring, as well as scoring of calli and somatic embryos were performed at the Central Biotechnology Laboratory (CBL). Floral buds of 4-5 mm long were harvested from the 6 cocoa hybrids once a week, early in the morning. They were used as sources of explants. Primary somatic embryos were obtained as described in Li *et al*. (1998), using staminode and petal explants onto 3 primary callogenesis media known as PCG1, PCG3 and PCG4. Fourteen days later, the culture onto Primary Callus Growth (PCG) medium, the callogenic explants were subcultured onto Secondary Callus Growth (SCG) medium. Fourteen days later, callogenic explants were again transferred onto hormone free Embryos Development (ED) medium. Callogenic explants were further subcultured three times, every 21 days onto the last medium.

A 8 x 2 x 3 factorial scheme in a modified completely randomised design was used for this study. Thus, 8 genotypes providing each 2 explants were cultured onto 3 culture media. Modifications were imposed on the design of the factor explants, namely staminodes and petals, of the same treatment, which were cultured on the same petri-dish and onto the same medium. Each treatment was prepared in triplicates

Phenological characters were observed during flower collection. Thus, fructification level was scored by counting cherelles, young and ripe fruits; whereas the flowering level and leaf flush were estimated according to a visual notation scale of 5 percentages, namely 0, 25, 50, 75 and 100. Thus, 0 corresponds to lack of floral buds and of new shoots on tree; 25, 50, 75 and 100% correspond to coverage rate of tree of 1/4, 1/2, 3/4 and 4/4 in floral buds as well as in new shoots.

At the end of 3 months, the number of callogenic explants, embryogenic explants, embryos number obtained per embryogenic explant were scored by counting. From these, the average embryos number per embryogenic explant as well as embryogenesis percentage, were calculated. The former was obtained by dividing the embryos number per embryogenic explant into the number of embryogenic explant. The latter was calculated by dividing the number of embryogenic explant into the number of callogenic explants.

SPSS and Xlstat, versions 16.0 and 2007, respectively were used for statistical analyses. The Principal Component (PCA), Hierarchical Cluster (HCA) and Factorial Discriminant Analyses (FDA) were performed using Wilks' Lambda method.

RESULTS

The PCA for the Measure of Sampling Adequacy (MSA) was 0.651 (Table 1). Such a value is considered to be average, according to Kaiser's scale, because it was between 0.6 and 0.7. The Bartlett's sphericity, postulating that at least one of the correlations between variables was significantly different from zero, was significant (approximate $\chi^2 = 63.006$; $P = 0.000$). The meeting of these two conditions made possible the performing of the PCA.

The number of callogenic explants and average number of embryos obtained per embryogenic explant made the Pearson's correlation matrix negative. This translated in a

TABLE 1. Eigenvalue, individual and cumulative variabilities and variable values on each of the components from the PCA

	F1	F2
Eigenvalue	4.085	1.241
Variability (%)	68.09	20.68
Cumulative %	68.09	88.77
Ncalem	0.978	0.178
Nemb	0.979	0.170
Pe	0.976	0.196
Nivflo	-0.049	0.946
Nivfru	0.482	0.631
Flush	0.574	0.710

null value of the determinant of this matrix and were thus dropped from the study. Consequently, the number of embryogenic explants, number of embryos per embryogenic explant, embryogenesis percentage, flowering level, fructification level and leaf flush were used in the rest of the study.

Out of the 6 principal components revealed by the PCA, only the first 2 displayed eigenvalues higher than 1 (Table 1; Fig. 1); thus, they met the Kaiser's criterion. They were selected to allow the interpreting of the variability expressed by the 6 hybrids analysed (Table 1). The 2 principal components explained 88.77% total variability; namely, component 1, accounted for 68.09% total variability. The Principal Component is defined by the number of embryogenic explants, embryos number yielded per embryogenic explant, embryogenesis percentage. It described hybrids yielding many somatic embryos (Table 1). Principal Component 2, described 20.68% of the unexplained variability by the component 1. It is determined by the flowering and fructification levels, as well as leaf flush. It characterised hybrids expressing a good phenological level (Table 1).

The projection of the calculated parameters on the principal plane from the PCA, displayed 2 groups; the first one, G1, was constituted by the flowering level, the second one, G2, was composed of the fructification level, leaf flush, number of embryogenic explants, number of embryos per embryogenic explant and embryogenesis percentage. Leaf flush and fructification level explained the number of embryogenic explants, embryos number obtained per embryogenic explant as well as embryogenesis percentage (Fig. 1).

With respect to the HCA, the number of individuals used was lower than 100 threshold, thus the HCA was chosen instead the k-means method (http://www.lemoal.org/spss/; accessed on August 25 th 2014). These individuals were separated into three clusters, consisted by 4, 2 and 2 individuals each, respectively. These individuals in clusters accounted for 50, 25 and 25%, respectively. Such percentages, highly greater than 10% level, authorised the validation of the analysis performed. Moreover, at level 10 of the scale of distance of the dendrogramme, truncation was done (Fig. 2). Such differences were showed by the Manova (P / Pillai's Trace =

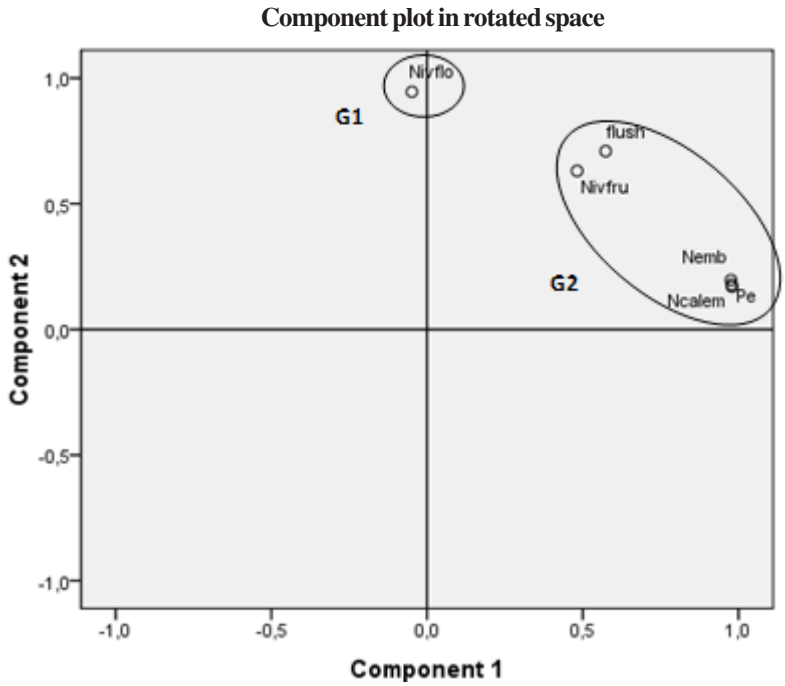

Figure 1. Scatter plot of measured variables on the principal plane from the Principal Component Analysis.

0.000; P / Wilks' Lambda = 0.000; P / Hotelling's Trace = 0.000; P / Roy's Largest Root = 0.000) to be very significant. Cross-examination of the data revealed that these differences resulted from 6 variables out of 8 used. Thus, 2 did not discriminate clusters, whereas 6 did, but partially (Fig. 2, Table 2).

Cluster C1 was composed of 4 individuals, namely L120-A2, L126-A3, L231-A4 and L330-A9. It was characterised by high number of callogenic explants, high flowering level, high fructification level and high leaves flush, but low number of embryogenic explants, low embryos number obtained per embryogenic explant, low average embryos number obtained per embryogenic explant and low embryogenesis percentage (Fig. 2; Table 2).

Cluster C2 consisted of 2 individuals, that is L232-A9 and L233-A4. It was marked by low numbers of callogenic explants, embryogenic explants, embryos per embryogenic explant, embryos yielded per embryogenic explant and embryogenesis percentage; and low flowering level, low fructification level and low leaves flush (Fig. 2, Table 2).

Cluster C3 consisted of 2 controls, namely C151-61 and SCA6. It was distinguished by high number of callogenic explants, embryogenic explants, embryos obtained per embryogenic explant, high average embryos number obtained per embryogenic explant and high embryogenesis percentage; as well as high flowering level, high fructification level and high leaves flush (Fig. 2; Table 2).

As for the FDA, the relationship between the 3 clusters and variables was assessed through the (i) looking for differences among clusters, (ii) validation of the Wilks' Lambda method, (iii) estimate of the coefficients of the discriminant function, and (iv) analysis of the representation quality. Thus, the number of callogenic explants, flowering level and leaf flush were selected to be

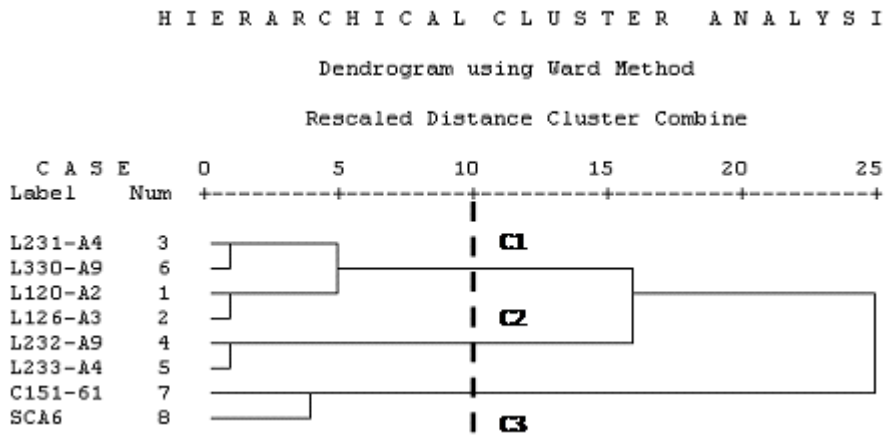

Figure 2. Partitioning of the 6 hybrid clones using all of callogenic, embryogenic and phenological parameters measured during the first year of the study.

TABLE 2. Classification of the cluster means from the HCA using Student-Newman-Keuls' test

Cluster	Ncal	Ncalem	Nemb	Mece	Pe	Nivflo	Nivfru	Flush
C1	13.832b	0.043a	0.115a	0.093a	0.217a	52.806a	17.806a	37.467b
C2	10.338a	0.013a	0.029a	0.024a	0.102a	34.049a	9.191a	24.474a
C3	13.161b	0.763b	3.592b	1.066b	3.856b	49.954a	24.846a	45.084b
Mean	12.444	0.273	1.245	0.394	1.392	45.603	17.281	35.675
P-value	0.033	0.000	< 0.0001	0.000	0.000	0.173	0.375	0.002

relevant for the analysis, on account of their very weak multicolinearity, compared to the other five. Indeed, the Variance Inflation Factor (VIF) of each of them was smaller than 10 threshold (VIF/Ncal = 2.381; VIF/Nivflo = 2.138; VIF/Flush = 1.880). Therefore, they were retained for the rest of the study. In contrast, those of five others was greater than 10 (VIF/Ncalem = 509.838; VIF/Nemb = 1460.050; VIF/Mece = 1090.770; VIF/Pe = 1500.214; VIF/Nivfru = 12.805). Consequently, Ncalem, Nemb, Mece, Pe and Nivfru were dropped from the study (Table 3).

Searching for the differences among identified clusters was achieved through means of calculated parameters on the one hand, and examination of Fisher-Snedecor F statistics and Wilks' Lambda on the other hand. Thus, regarding the means, those of the 3 parameters seemed discriminant (Ncal = 163.605; Nivlfo = 2247.130; Flush = 1304.886). Regarding the Fisher-Snedecor's F statistics, it was high (Ncal = 24.771; Nivflo = 2.576; Flush = 12.466). As for the Wilks' Lambda, that of the 3 parameters was smaller or equal to 0.9 (Ncal = 0.092; Nivflo = 0.493; Flush = 0.167). Thus, analysis of the 3 aforementioned criteria displayed the existence of differences among 3 clusters identified.

The validation of the Wilks' Lambda method was done through the Box's M statistics, global correlation and Wilks' Lambda. Globally, stepwise statistics revealed that it was possible to extract from 3 initial parameters found to be relevant, only 2 containing sufficient information allowing for complete discrimination of the 3 previously identified clusters. These are, hierarchically, first the number of callogenic explants, then leaves flush. Indeed, in step 1, the entry of the number of callogenic explants induced discrimination of the clusters (l = 0.092; P = 0.003). Similarly, addition of the leaf flush to the number of callogenic explants, in the second step of the analysis, also triggered discriminating of clusters (l = 0.022; P = 0.002). More specifically, for the Box's M statistics, the variance-covariance matrixes were statistically equal (Box's M = 1.855; P = 0.241), implying the choice of a linear FDA. The global correlations tended towards 1, namely 0.979 and 0.687 for the discriminant functions 1 and 2, respectively. The discriminant function 1 allowed in the proportion of 96.20% the discriminating of clusters, as against 3.80% for the function 2. The Wilks' Lambda was equal to 0.022 and 0.528 with P corresponding to 0.02 and 0.090, respectively. The Wilks' statistcs only allowed the validation of the function 1.

Clusters C1 and C3 were placed in the positive part of axis F1, while cluster C2 was placed in the negative part (Fig. 3). C1 and C3 were linked with high calli production and leaves flush, whereas C2 was associated with the low ones (Table 2). From these, 2 discriminant functions were extracted as represented by equations1 and 2:

$$Z1 = -29.123 + 0.201*Flush + 1.71*Ncal \quad \text{..... Eq. 1}$$

$$Z2 = 1.202 + 0.054*Flush - 0.628*Ncal \quad \text{..... Eq. 2}$$

Only the first one completely discriminated clusters as reported previously.

The representation quality was appreciated *via* the confusion matrix. It showed that in class C1, 75% individuals represented by hybrids L126-A3, L330-A9 and L120-A2 were well-classified thanks to the discriminant function 1. In contrast, 25% individuals represented by hybrid L231-A4 were badly classified. Likewise, in clusters C2 and C3, 100 and 100% individual were correctly classified, respectively (Table 4).

However, in step 1, the F statistics for pairwise distance, calculated between clusters C1 and C2, introducing the number of callogenic explants in the analysis, was significant (P =

TABLE 3. Detecting of uncorrelated parameters *via* their Variance Inflation Factor (VIF)

Statistics	Ncal	Ncalem	Nemb	Mece	Pe	Nivflo	NivfruFlush	
Tolerance	0.111	0.002	0.001	0.001	0.001	0.255	0.078	0.532
VIF*	9.007	509.838	1460.050	1090.770	1500.214	3.928	12.805	1.880

* = Variance Inflation Factor calculated from formula 1 / Tolerance. The latter itself is calculated from formula 1 – R^2, where R^2 represents the coefficient of determination expressing the fit degree of the data to model

TABLE 4. Assessment of the representation quality by means of the confusion matrix

		Ward Method	Predicted group membership			Total
			C1	C2	C3	
Original	Count	C1	3	0	1	4
		C2	0	2	0	2
		C3	0	0	2	2
	%	C1	75	0	25	100
		C2	0	100	0	100
		C3	0	0	100	100

TABLE 5. Comparison of the clusters used in the analysis

Step	Ward Method*	C1	C2	C3
1	C1	F*	48.453	1.787
		p-value*	0.001	0.239
	C2	F	48.453	23.724
		p-value.	0.001	0.005
	C3	F	1.787	23.724
		p-value	0.239	0.005
2	C1	F	39.528	1.862
		p-value	0.002	0.268
	C2	F	39.528	32.673
		p-value	0.002	0.003
	C3	F	1.862	32.673
		p-value	0.268	0.003

Ward Method* : F* = Fisher and Snedecor's statistics calculated for pairwise distances. p-value* = Observed probability to compare to the theorical one, namely 5%

0.001). Following the same context, the distance between C2 and C3 was significant (P = 0.005). In contrast, the distance between C1 and C3 was not significant (P = 0.239). In step 2, addition of the leaves flush to the number of callogenic explants provided similar results to the previous ones. The distances between C1 and C2 as well as C2 and C3 were significantly different (P = 0.003). However, the distance between C1 and C3 was not significant (P = 0.268). Consequently, there were 2 clusters rather than 3. First, represented by clusters C1-C3, was composed of 4 hybrids L120-A2, L126-A3, L231-A4, L330-A9 as well as control clones SCA6 and C151-61. Second, represented by cluster C2, was constituted of hybrids L232-A9 and L233-A4 (Table 5; Fig. 3).

DISCUSSION

Variability of the *in vitro* and phenological behaviours of 6 newly developed cocoa hybrids were analysed using the discriminant analysis. In Issali *et al.* (2011), the typology provided from all of callogenesis and somatic embryogenesis parameters used here, but without using the phenological parameters, the results were similar to those reported here. From this, hybrids L233-A4 and L126-A3 belonged to different clusters. This was also true in the present study. Our work shows that the 4 hybrids used in this study expressed the same callogenic and phenological behaviours.

The number of embryogenic explants, number of embryos per embryogenic explants,

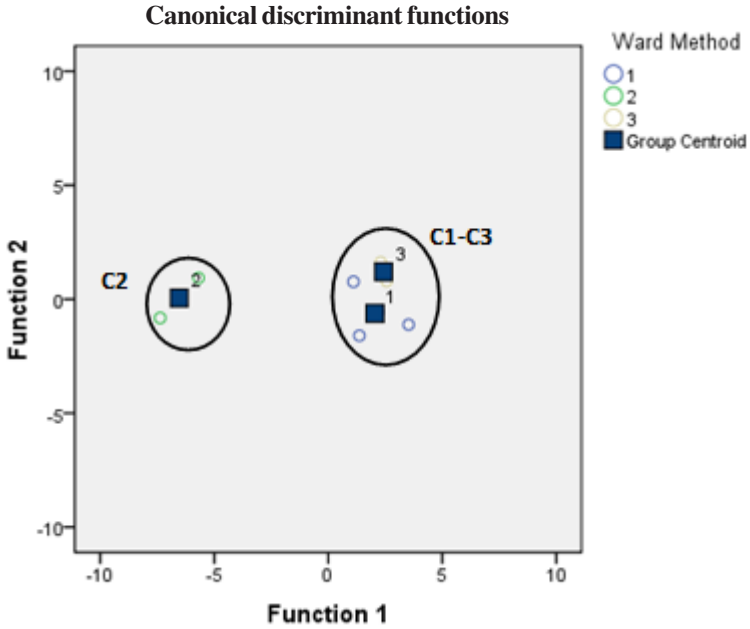

Figure 3. Factorial map showing the 3 clusters formed from the 6 hybrids using the FDA during the first year of the study.

embryogenesis percentage, flowering level, fructification level and leaves flush were relevant for the PCA (Fig. 1, Table 1). Consequently, the number of callogenic explants and average number of embryos per embryogenic explant were dropped from the analysis. Indeed, the latter made the Pearson's correlation matrix negative and blocked the outputting of some results. In contrast, for the FDA, the number of callogenic explants and leaf flush were found relevant. For this purpose, use of Variance Inflation Factor appears to be necessary to detect and select relevant parameters intended to be used in the FDA. If not, non-discriminant parameters might be part of the analysis and thus, make the latter uninterpretable. Therefore, the relevance of variables seems to vary as a function of the analysis method.

In the same way, fructification level and leaf flush~~es~~ explained somatic embryogenesis (Fig. 1). Thus, fluctuations in the fructification level and leaf flush induced somatic embryogenesis variations. Such a relationship was reported in Issali *et al.* (2009), but using the Pearson's linear correlations. This can be explained through some internal fluctuations of the plant growth regulators, such as indole-3-acetic acid and/or gibberellic acid, induce the phenological characters (Kofler, 1969). The latter, in turn, influence the callogenesis and somatic embryogenesis expressions. Therefore, cocoa tissue culture for somatic embryogenesis purposes could be achieved when leaf flush is high.

Four cocoa hybrids, namely L120-A2, L126-A3, L231-A4, L330-A9, belonging to clusters C1-C3 yielded the highest number of callogenic explants and expressed the highest level of leaf flush (Fig. 3). It was the same for controls SCA6 and C151-61. Concerning hybrids, the first 3 are half sibs of common male parent IMC67 (Issali, 2012). The latter was found to be the most embryogenic (Minyaka *et al.*, 2008). Following the same idea, clone C151-61 comes from a back cross ICS1 x (ICS1 x SCA6) as reported in Lockwood and Gyamfi (1979). Thus, clone C151-61 comes from SCA6; the latter is one of the donor parents of C151-61. The 4 hybrids in the present study, could be used to produce chocolate aroma, cocoa butter and theobromin from the calli suspensions in bioreactors. Regarding C2, L232-A9 and L233-A4 belong to cluster C2, are half sibs of common male parent Pa150 (Issali, 2012); (Table 5; Fig. 3). Indeed, the former comes from cross Pa13 x Pa150, while the latter is from Pa121 x Pa150. Thus, their similar behaviour might be

explained by the common genetic identity from parent Pa150. We could benefit from breeding them before improving their yielding. Consequently, staminodes and petals extracted from floral buds belonging to these hybrids should be *in vitro* cultured when leaf flush is high. Indeed, somatic embryogenesis and leaves flush were positively correlated (Data not shown). The first discriminant function (equation 1) was the best between the 2 proposed by the output . As far as the 4 above-mentioned cocoa hybrids are concerned, this equation shows that the discrimination scores Z1 ranged from -0.636 to 5.166 for individuals belonging to cluster C1-C3. Consequently, an individual expressing a given score, when included in this values rank, will display high callogenesis and leaf flush. In contrast, for the 2 others hybrids, namely L232-A9 and L233-A4, their discrimination scores stretched out from -7.628 to -5.421 for individuals belonging to cluster C2 (Fig. 3). Individuals which express such discrimination scores will yield low callogenic explants and leaf flush. Equation 1 allows for prediction of a new individual belonging to a cluster from its values on condition that its number of callogenic explants and leaf flush are known. It also allows for better discrimination and description of the clusters identified. Therefore, instead of 3 clusters, the 6 studied cocoa hybrids were structured into 2 clusters using the multivariate analysis, namely the FDA.

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