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Research Article

DIVERSITY IN THE *IN VITRO* AND PHENOLOGICAL RESPONSES OF 6 COCOA HYBRIDS USING THE DISCRIMINANT CLASSIFICATIONS FOR 2 YEARS OF THE STUDY

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Abstract: To study the diversity in the *in vitro* and phenological responses, the model discriminant was used. Six new hybrid cocoa genotypes namely L120-A2, L126-A3, L231-A4, L232-A9, L233-A4 and L330-A9 were analyzed. Three culture media namely PCG1, PCG3 and PCG4, based on DKW basal medium, only differing in hormonal concentration, were sown with staminodes and petals extracted from floral buds. SCA6 and C151-61 were used as controls. The 6 cocoa hybrids were assessed 3 months later by scoring and calculating of the callogenesis and embryogenesis variables. Parameters from variable means were processed via the Principal Component (PCA), Hierarchical Cluster (HCA) and Factorial Discriminant Analyses (FDA). For the PCA, the number of embryogenic explants, embryos number yielded per embryogenic explants, embryogenesis percentage, flowering level, fructification level and leaves flush were revealed to be pertinent. It was the same in the first year of the study. However, for the FDA, only the number of callogenic explants and leaves flush were showed to be pertinent. This indicates that the pertinentness appears to vary from analysis to analysis. Cocoa hybrids known as L120-A2, L126-A3, L231-A4 and L330-A9 yielded the highest number of callogenic explants and expressed the highest leaf flush. The first 3 are half sibs of common father IMC67, justifying their belonging to the same group. The discriminant model associated with the analysis is spelt Z1 = - 29.123 + 0.201*Flush + 1.710*Ncal. It significantly and completely discriminated the clusters in the proportion of 96.20%. The predicting of the membership cluster of new observations, from their number of callogenic explants and leaves flush, was discussed.
Keywords: Callogenesis; Callus growth media; Leaves flush.
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INTRODUCTION

Cocoa tree is a bush which grows in the natural rainforest of tropical America (Braudeau, 1969; Mossu, 1990). Its dried seeds named beans are used in manufactures to provide finished or semi-finished products such as chocolate biscuit, soap and cosmetic factories as well as perfumery, among others (Braudeau, 1969). Côte d'Ivoire is the largest cocoa producer in the world with yearly average yielding of 1.200.000 tons, corresponding to 41% world supply. Cocoa area covers about 2,176,000 ha accounting for 6% of national area. Out of 16,000,000 people, 6,000,000 directly or indirectly live of cocoa. This accounts for 37.5%. Cocoa cultivation yields about 30% of global export revenue. It participates in more than 15% at gross domestic product (ICCO, 2000). Moreover, the average productivity in beans, in cocoa farms, in the order of 400-800 kg / ha, are low (Mossu, 1990). In contrast, in research station, this productivity reaches 2.5 tons / ha (Mossu, 1990). One of the means to improve it is the creation of new varieties. In 1988, 28 cocoa

off-springs were created and planted in field C2/1 in the ancient field of the CNRA (Centre National de Recherche Agronomique) located at Bingerville (N'goran, 1988). Thirty individuals were preselected. Among them, 6 were proposed to the *in vitro* culture unit of the Central Biotechnology Laboratory (CBL) from CNRA. This, to clone them via SE. These are L120-A2, L126-A3, L231-A4, L232-A9, L233-A4 and L330-A9 from which staminodes and petals were extracted and used as explants source. Simultaneously, during floral buds collection, 3 phenological variables were weekly recorded. These are flowering and fructification levels as well as leaves flush. These cocoa hybrids were already characterized using an univariate approach (Issali *et al.,* 2008; Issali, 2011a,b; Issali, 2012). Such a characterization was achieved in relation to callogenesis and somatic embryogenesis (SE). Following the same idea, the relationship between 3 phenological parameters and SE was also assessed, but always using only one embryogenesis parameter (Issali et al., 2009). No multivariate typology, both based on the means of callogenesis, SE and phenology recorded for 2 years of the study, was not yet performed.

Nonetheless, from the data of the first and second year, individually taken, the variability of the *in vitro* and phenological behaviours of 6 cocoa hybrids was analyzed (*Issali et al.,* 2014a, b). Indeed, in the first year, 4 hybrids out of 6, namely L120-A2, L126-A3, L231-A4 and L330-A9, both expressed the highest callogenesis and leaf flush values. In the same vein, the leaves flush was positively correlated with the 4 embryogenesis parameters. This suggested that explants from these hybrids should be cultured *in vitro* when leaves flush would be high. In contrast, in the second year, hybrids L120-A2 and L126-A3 expressed the highest callogenesis and flowering level compared to 4 others. Here, on the contrary, leaves flush was positively correlated with callogenesis parameter postulating that the callogenesis would be optimized when cultured explants would proceed from a tree bearing many awake axillary buds. Thus, to date, the diversity of the *in vitro* and phenological responses, of 6 cocoa hybrids simultaneously related to callogenesis, SE and phenology for 2 years, from average values, has stayed unknown. On account of the weak variation of the climatic variables in intertropical region, the structuring outputted might be similar to that of one of the 2 years of the study, individually taken. Indeed, the climatic variations influence the phenological variations, and thus internal plant growth regulator variations (Braudeau, 1969; Augé et al., 1984; Issali, 2010). The climate of some years is often alike. The concomitant analysis of these new cocoa hybrids based on 8 callogenesis, somatic embryogenesis and phenology parameters could allow better describing them. Likewise, it will allow the identifying of the phenological phase which optimizes callogenesis and somatic embryogenesis. The structuring of this diversity could allow the explaining of the *in vitro* and phenological behaviours. Likewise, it could allow the predicting of membership group of a new observation. The goal of the present work was to structure the diversity of the *in vitro* and phenological responses of 6 new cocoa hybrids.

EXPERIMENTAL

Plant materials, experimental sites and design

Plant materials were composed of 6 new hybrids preselected for yielding and resistance to Phytophthora pod rot. These are L120-A2, L126-A3, L231-A4, L232-A9, L233-A4 and L330-A9. They were created 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, while the last 3 are half sibs with Pa150 as common male parent. Trees were planted according to a completely randomized design in three geographical zones namely C2/1 of the ancient station of CNRA. Clones SCA6 and C151-61 were used as controls. The former was identified like very embryogenic, while the latter descends from back cross 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, Côte d'Ivoire. Forty trees were planted per progeny. A border composed of 138 trees was associated with the design. A gap of 2.5 m was maintained among trees on the same row,

while the one among rows was of 3 m. This corresponds to density of 1333 trees/ha. The experiment was carried for 2 years. Here, the means from collected data for 2 years were used. Thus, the experiment regarding tissue culture took place, in the first year, from September 2002 to August 2003. In the second year, the experiment stretched out from January to December 2004.

The work was carried out in two experimental sites. These are floral bud collection and tissue culture sites. The collection site of floral buds was represented by the ancient station of CNRA was located at Bingerville. This site was located at 3°52'59" West and 5°21'42" North. During the 2 years of the study, the weekly pluviometric total, weekly average maximum temperature, weekly average minimum temperature, sunshine average relative humidity were 4186.80 mm, 30.63 °C, 20.16°C, 77651.42 hours, 82.13%, respectively. The second site for tissue culture was represented by the CBL. Cultures initiation and monitoring as well as scoring of calli and somatic embryos were performed there. Floral buds measuring 4-5 mm long were harvested on the 6 cocoa hybrids once a week, early in the morning. They were used as a source of explants. Primary somatic embryos were obtained as described by culturing of staminode and petal explants onto 3 primary callogenesis media known as PCG1, PCG3 and PCG4. Fourteen days later the culture onto PCG, the callogenic explants were sub-cultured onto Secondary Callus Growth (SCG). Fourteen days later, callogenic explants were again sub-cultured onto hormone free Embryos Development medium (ED). Onto the latter, callogenic explants were subcultured thrice every 21 days. At CBL, a 8 x 2 x 3 factorial combination in a modified completely randomised design was used. Thus, 8 genotypes providing each 2 explants were cultured onto 3 primary callogenesis media. Modifications concerned explants, namely staminodes and petals, of a same treatment which were cultured in the same Petri dish onto the same medium. Each treatment was prepared in triplicate. In all, 72 treatments were obtained at the end of a weekly culture initiation.

Measured variables

Regarding tissue culture, at the end of 3 months, the number of callogenic explants, number of embryogenic explants, embryos number yielded per embryogenic explants was scored. From these, the average embryos number yielded per embryogenic explants as well as embryogenesis percentage were calculated. In the analyses, means from these 8 variables were used. As far as the phenological characters are concerned, they were observed during flowers collecting. The fructification level was scored. In contrast, the flowering level and leaves flush were estimated according to a visual notation scale of 5 percentages, namely 0, 25, 50, 75 and 100.

Statistical Analysis

The collected data were processed under software SPSS and Xlstat, versions 16.0 and 2007, respectively. The Principal Component (PCA), Hierarchical Cluster (HCA) and Factorial Discriminant Analyses (FDA) were performed to interpret the diversity. As for the PCA, the method varimax with Kaiser's normalisation was achieved. Regarding the HCA, the squared euclidian distance was achieved for distances calculation, while the average linkage between groups was used as an aggregation option. For the FDA, Wilks' Lambda was used.

RESULTS

Data compressibility through the PCA

The KMO (Kaiser-Meyer-Olkin) coefficient was 0.651. Such a coefficient is noted to be average, after Kaiser's scale, because it is between 0.6 and 0.7. The Bartlett's sphericity, suggesting that at least one of the correlations between parameters is significantly different from zero, was significant (approximate $\chi^2 = 63.006$; p-value = 0.000). The meeting these 2 conditions allowed the performing of the PCA. The number of callogenic explants and average embryos number per embryogenic explant made null the determinant of the Pearson's linear correlation matrix. Therefore, they were dropped from the study. Thus, the numbers of embryogenic explants, embryos number yielded per embryogenic explant, embryogenesis percentage, flowering level, fructification level as well as leaves flush were used in the rest of

analysis. Out of 6 factorial axes obtained from the linear combination of the parameters measured, sole the first 2 expressed eigenvalues higher than 1. Thus, they met the Kaiser's criterion. Consequently, they were used in the rest of the study to interpret the variability expressed by the 6 cocoa hybrids (Table 1).

The plane 1-2, composing the first 2 axes, accounted for 88.77% total variability. First, namely F1, described 68.09% total variability. It is defined by the number of embryogenic explants, embryos number yielded per embryogenic explant and embryogenesis percentage. It characterised the cocoa hybrids expressing good ability to SE (Table 1). Second, coded F2, showed 20.68% of the unexplained variability by the axis F1. It is determined by the flowering and fructification levels as well as leaves flush. It marked the cocoa hybrids expressing good phenology (Table 1).

The projection of the previously identified parameters on the principal plane from the PCA displayed 2 clusters. The first one was constituted of the flowering level. The second one was composed of the fructification level, leaves flush, number of embryogenic explants, embryos number yielded per embryogenic explant, embryogenesis percentage. In sum, the fructification level and leaves flush explained the number of embryogenic explants, embryos number yielded per embryogenic explant as well as embryogenesis percentage (Figure 1).

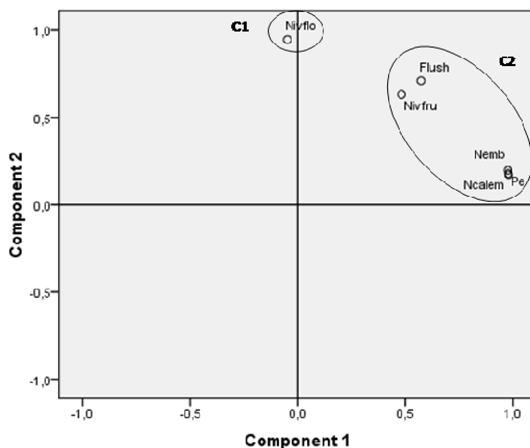

Figure 1. Clustering of the calculated parameters on the principal plane 1-2 from the PCA

Table 1. Synthesis on each component of the eigenvalue, variation percentage and parameters value 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**

Diversity of the *in vitro* and phenological responses of the 6 cocoa hybrids for 2 years of the study via the HCA

The number of observations used in the study was equal to 8. This number, being lower than 100, allowed the choice of the HCA instead k-means analysis (http://www.lemoal.org/spss/, accessed on August 20 th 2014). These observations were initially truncated into 3 clusters, consisted of 4, 2 and 2 individuals each, representing 50, 25 and 25%, respectively. Such percentages, widely greater than 10% level, in each cluster, allowed the validation of the analysis performed. At level 10 from the dissimilarity scale of the dendrogramme, the partitioning was done. Such significant variations were displayed through the Manova (p-value / Pillai's Trace = 0.000; p-value / Wilks' Lambda = 0.000; p-value / Hotelling's Trace = 0.000; p-value / Roy's Largest Root = 0.000). The data examination revealed that, out of 8 calculated parameters, 2 did not discriminate the 3 identified clusters. These are the flowering and fructification levels. However, 6 partially discriminated the 3 clusters identified. These are the number of callogenic explants, number of embryogenic explants, embryos number yielded per embryogenic explant, average embryos number per embryogenic explant, embryogenesis percentage and leaves flush (Table 2 and Figure 2).

Thus, cluster C1 consisted of 4 observations, especially L120-A2, L126-A3, L231-A4 and L330-A9. It was marked by high number of callogenic explants, high flowering level, high fructification and high leaves flush, but low number of

embryogenic explants, embryos number yielded per embryogenic explants, low average embryos number per embryogenic explant and low embryogenic percentage (Table 2).

Cluster C2 comprised 2 individuals, in particular L232-A9 and L233-A4. It differed from the first one by low number of callogenic explants, low number of embryogenic explants, low embryos number yielded per embryogenic explant, low average embryos number per embryogenic explant, low embryogenic percentage, low flowering level, low fructification level and low leaves flush (Table 2). Cluster C3 was constituted of 2 control clones, notably C151-61 and SCA6. It stood out from the first 2 prior by high number of callogenic explants, high number of embryogenic explants, high embryos number yielded per embryogenic explant, high average embryos number per embryogenic explant, high embryogenic percentage, high flowering and fructification levels as well as high leaves flush (Table 2).

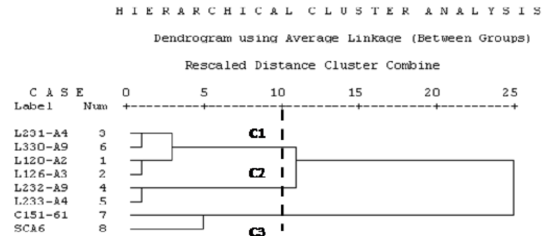

Figure 2. Hierarchical tree displaying the partition of 6 analysed cocoa hybrids using the tissue culture and phenology parameters.

Table 2: Variation of the means of clusters outputted by the HCA using the 8 parameters according to Student-Newman-Keuls' test

Cluster	Ncal	Ncalem	Nemb	Mece	Pe	Nivflo	Nivfru	Flush
C1	13.832a	0.043a	0.115a	0.093a	0.217a	52.806a	17.806a	37.467a
C2	10.338a	0.013a	0.029a	0.024a	0.102a	34.049a	9.191a	24.474b
C3	13.161b	0.763b	3.592b	1.066b	3.856b	49.954a	24.846a	45.084b
Mean*	12.444	0.273	1.246	0.394	1.391	45.603	17.281	35.675
F	24.771	203.128	543.483	193.285	711.134	2.576	2.965	12.466
p-value	0.003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.170	0.142	0.011

Mean* : It was calculated from values of the clusters C1, C2 and C3.

Relationship between the 3 clusters and 8 calculated parameters using the FDA

This relationship was analysed via the : 1) searching for the differences among clusters, 2) validation of the Wilks' Lambda method, 3) assessing of the discriminant power of axes and 4) the analysis of the representation quality. Prior, the relevant parameters were searched for choosing the ones recording Variance Inflation Factor (VIF) smaller than 10. Thus, the number of callogenic explants, embryos number yielded per embryogenic explant, average embryos number yielded per embryogenic explant, embryogenesis percentage and fructification level expressed VIF higher than 10 (VIF / Ncalem = 509.838; VIF / Nemb = 1460.050; VIF / Mece = 1090.770; VIF / Pe = 1500.214; VIF / Nivfru = 12.805). They were dropped from the study. In the opposite, the number of callogenic explants, flowering level and leaves flush recorded VIF smaller than 10 (VIF / Ncal = 9.007; VIF / Nivflo = 3.928; VIF / Flush = 1.880). They were used in the rest of the study (Table 3).

Concerning the existence of differences among the 3 clusters, only the number of callogenic explants and leaves flush seemed to be the most discriminant (Mean / Ncal = 12.791; Mean / Flush = 36.123). About Fisher-Snedecor' F statistics, the analysis of equality test among mean groups confirmed the suspicion (F / Ncal = 24.771, p-value = 0.003; F / Nivflo = 2.576, p-value = 0.170; F / Flush = 12.446, p-value = 0.011). Concerning Wilks' Lambda, that of these 2 parameters were smaller or equal to 0.9 with the same aforementioned p-values (λ / Ncal = 0.092; λ / Nivflo = 0.493; λ / Flush = 0.167). Therefore, the examination of these 3 criteria showed the existence of differences among the clusters evidenced. With respect to the validation of the Wilks' Lambda approach, the global correlation as well as Wilks' Lambda was examined. The Wilks' Lambda for the test of the discriminant functions showed that the first one recorded Lambda very weak and significant (λ / function 1= 0.022; p-value = 0.002). However, the second discriminant function was certainly low,

but insignificant (λ / function 2 = 0.522; p-value = 0.090). Consequently, the second discriminant function was not taken into account, while the first one was it. The stepwise technique displayed that it was possible to extract from 3 precedent evidenced parameters, only 2 parameters that partially discriminated the 3 clusters (Table 2). These are the number of callogenic explants and leaves flush. The linear discriminant function 1, consisted of the number of callogenic explants and leaves flush, discriminated in proportion of 96.20% the 3 clusters evidenced. Thus, the Wilks' Lambda approach was only validated for the first discriminant function. The clusters C1-C3 were put in the positive part of canonical axis F1, while the cluster C2 was placed in the negative one (Figure 3). The C1-C3 recorded the highest number of callogenic explants and leaves flush. In contrast, the cluster C2 recorded the lowest ones (Table 2; Figure 3). The linear discriminant models, associated with the study, are written Z1 = - 29.123 + 0.201*Flush + 1.710*Ncal and Z2 = 4.417 + 0.168*Flush – 0.820*Ncal, for the first and second function, respectively. The first function Z1 discriminated the clusters in the proportion of 96.20%, as against 3.80% for the second one. Sole the first function was validated. It showed that in the equation Z = Z0 + W1X1 + W2X2, the intercept for discrimination score Z0 is equal to–29.123; the first discrimination weight W1 is equal to 0.201. It was associated with predictor leaves flush. The second discrimination weight W2 is equal to 1.710. It was linked with predictor number of callogenic explants. The values Z0, W1 and W2 are the estimates of coefficients of the discriminant function 1. The examination of the representation quality was done via the confusion matrix. This revealed that in cluster C1, 75% observations were correctly reclassified. In contrast, 25% observations were not correctly reclassified. In clusters C2 and C3, 100% observations were well-reclassified. This, thanks to the discriminant function 1 (Table 4). In the first step, the introduction of the number callogenic explants in the analysis, in sight of to calculate pair wise distance C1 and C2, showed that the latter was significant (F = 48.453; p-value = 0.001). Following the same idea, the pairwise distance between clusters C1 and C3, was not significant (F = 1.787; p-value = 0.239; Table 5). In the second step, the adding of the leaves flush in the analysis revealed that the same distance between C1 and C2 has stayed significant (F = 39.528; p-value = 0.002). In the same vein, between C1 and C3, the distance has remained insignificant (F = 1.862; p-value = 0.268). In brief, these 3 previously identified clusters did not constitute 3 morphologically distinct entities. Only the clusters C1-C3 and C2 formed morphologically distinct entities (Table 5, Figure 3).

Table 3. Statistics of Multicolinearity Calculated from the 8 Parameters used

Statistics	Ncal	Ncalem	Nemb	Mece	Pe	Nivflo	Nivfru	Flush
Tolerance	0.111	0.002	0.001	0.001	0.001	0.255	0.078	0.000
VIF	9.007	509.838	1460.050	1090.770	1500.214	3.928	12.805	1.880

VIF*: 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 Representation Quality via Confusion Matrix

			Predicted Cluster Membership			
	Average Linkage (between clusters)		C1	C2	C3	Total
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. Matrix Revealing Comparison and Significance of the Pair-wise Distances among 3 Clusters Presumed

Step	Parameter intoduced In the analysis	Average Linkage (between clusters)*		C1	C2	C3
1	Ncal	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	Ncal Flush	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	

Average link (between clusters)*: F* : Fisher and Snedecor' s statistics calculated for pairwise distances.
p-value* :Calculated probability to compare to that of 5%.

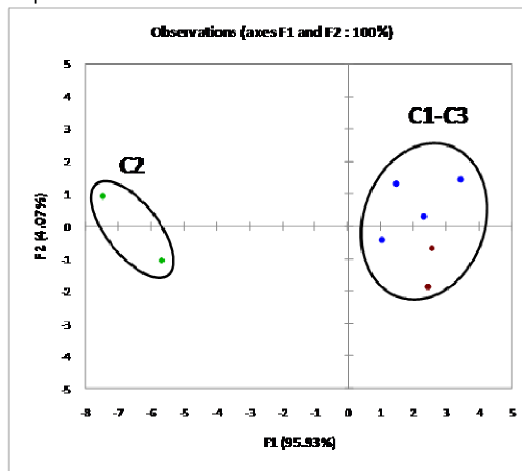

Figure 3. Factorial map illustrating the 2 clusters formed by the 6 cocoa hybrids using FDA for 2-year of the study

Average link (between clusters): F* : Fisher and Snedecors statistics calculated for pair-wise distances.
p-value* : Calculated probability to compare to that of 5%.

DISCUSSION

The study aiming the analysis of the diversity of the *in vitro* and phenological responses of 6 cocoa promising hybrids was carried out. The present study is the synthesis of the data collected for 2 years. Previously, the same analysis was achieved during the first and second year of the study (Issali *et al.,* 2014a and b). Thus, in the first year, 2 clusters were identified, as against 3 in the second one. In the present synthetical study reported here, only the structuring during the first year of the study corresponded to the one including the mean of 2 years of this study. The number of embryogenic explants, embryos number yielded per embryogenic explant, embryogenesis percentage, flowering level, fructification level and leaves flush, were revealed to be pertinent relatively to the PCA (Figure 1, Table 1). During the first and second year of the study, the same parameters were found to be pertinent. Therefore, the number of callogenic explants and average embryos number yielded per embryogenic explant were dropped from the study. The former was scored, while the latter was calculated. They did not allow not only the calculation of determinant of the Pearson's linear correlation matrix, but also they reduced the percentage of the variation explained by the first 2 components. Thus, the data from the first and second year as well as their means represented

by those analyzed here expressed the same factorial structure. As for always the pertinentness of used parameters, in general, those evidenced in the PCA was different from those from the FDA. Sure enough, regarding the FDA, in the first year like in the present analysis, 3 parameters were found common and pertinent. These are the number of callogenic explants, flowering level and leave flush (Issali *et al.,* 2014a). In the second year, the number of callogenic explants, flowering level and fructification level were displayed to be pertinent (Issali, 2011b). This proved that the parameters responsible for multicolinearity, thus perfect correlations, varied from year to year. The multicolinear parameters hinder the performing of some calculations such as the matrix calculation. The average data deriving from 2 years of the study seems to suit for the structuring of the variability of the *in vitro* and phenological responses. The leaves flush and fructification level explained the number of embryogenic explants, embryos number yielded per embryogenic explant and embryogenesis percentage (Figure 1). The SE is an induced phenomenon (Duhem *et al.,* 1989). The exogenous and endogenous plant growth regulators are responsible for that (Augé *et al.,* 1984; Alemanno, 1995). The phenological cycles are governed by the internal fluctuations of the plant growth regulators (Heller et al. 1995). Thus, these 5 parameters are correlative. Such a correlation would be due to pleiotropic genes (Baradat, 1986). Four cocoa hybrids especially L120-A2, L126-A3, L231-A4 and L330-A9 as well as the 2 control clones C151-61 and SCA6 displayed the highest number of callogenic explants and leaves flush (Figure 2; Table 2). This result is similar to the one obtained in the first year of the study (Issali *et al.,* 2014a). Therefore, the mean is a good estimator of the individual measured values. Indeed, the first 3 cocoa hybrids descended from male parent IMC67 (Issali, 2012). Thus, they are half sibs with IMC67 as a common male parent. Nonetheless, hybrid L330-A9 comes from male parent Pa150. This justified the belonging of these first 3 to the same cluster. Following the same idea, the control clones SCA6 and C151-61 displayed the highest number of callogenic explants and flowering level. Control clone C151-61 descends from back cross ICS1 x (ICS1 x SCA6) as reported in Lockwood and Gyamfi (1979). Thus, clone SCA6 is one of the male parents of clone C151-61. These 4 previous hybrid genotypes could be used to produce chocolate aroma, cocoa butter and the obromin from the calli suspensions in bioreactors for biotechnology purposes. Sole the linear discriminant function spelling $Z1 = -29.132 + 0.201^{*} Flush + 1.710^{*}Ncal$ significantly and completely discriminated the 2 clusters identified. In contrast, $Z2 = 4.417 + 0.168^{*}Flush - 0.820^{*}Ncal$ did not discriminate them (Table 5; Figure 3). This equation revealed that, high producing genotypes of calli, will express a number of callogenic explants ranging from 12.861 to 14.597. In the same vein, they will show a leaves flush level varying from 32.56 to 46.40%. Therefore, their Z1 will fluctuate from 2.188 to 5.156. This, for genotypes belonging to cluster C1-C3. In contrast, low producing genotypes will display a number of callogenic explants oscillating from 10.261 to 10.415. Their leaves flush will fluctuate from 19.63 to 29.31%. Consequently, their Z1 will vary from – 7.638 to – 5.430. This, for genotypes belonging to cluster C2 (Figure 3). Such an approach will allow the predicting of membership group of a new observation from its number of callogenic explants and leaves flush values.

CONCLUSION

We have made the assumption that the structuring of 6 cocoa hybrids into 2 morphologically distinct clusters using the means from 2 years was similar to that of the first year of the study. Effectively, The 2 clusters C1-C3 and C2 as well as their composition, in the first year as well as in the 2 years of the study, were the same. Therefore, we can use the means derived from several years of the study of the diversity analysis for structuring purposes. Cocoa hybrids, from cluster C1-C3, known as L120-A2, L126-A3, L231-A4 and L330-A9, yielded the highest number of callogenic explants and expressed the highest leaf flushes. Their staminode and petal explants must be cultured in vitro when the leaves flush will be high. The linear equation $Z1 = -29.123 + 0.201^{*}Flush + 1.710^{*}Ncal$ modelled the discriminating of clusters as a function of the

number of callogenic explants and leaves flush. Thus would hope us to increase the callogenesis culturing staminodes and petals when leaves flush would be high. This equation allowed the prediction of membership cluster of a new individual from its callogenesis and leaf flush values.

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