ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 7 (1): 51-58, 2008
ISSN 1682-296X

Characterization of Callogenic and Embryogenic Abilities of Some Genotypes of Cocoa (*Theobroma cocoa* L.) Under Selection in Côte d'Ivoire

[1,2]Auguste Emmanuel Issali, [3]Abdoulaye Traoré, [1]Edmond Kouablan Koffi,
[1]Jeanne Andi Kohi N'goran and [1,2]Abdourahamane Sangaré
[1]Laboratoire Central de Biotechnologies,
Centre National de Recherche Agronomique, 01 BP 1740 Abidjan 01, Côte d'Ivoire
[2]Laboratoire de Génétique, UFR Biosciences,
Université de Cocody/Abidjan, 22 BP 582 Abidjan 22, Côte d'Ivoire
[3]The School of Forest Resources, Pennsylvania State University,
410 Life Sciences Building, University Park, PA 16802,

Abstract: To optimize the somatic embryogenesis, a characterization *in vitro* by an improved process of some cocoa genotypes was undertaken. Six hybrids, five parents and two clones as control were used. Staminodes and petals were cultured on three primary callus growth media (PCG) differing in hormonal balance 2.4-D/TDZ. Two primary descriptors (callogenic and embryogenic explant number) and three secondary descriptors (general abilities, favourable explants and PCG media) were used to characterize studied genotypes. The separate characterization of factors from two primary descriptors, allowed identifying of four hybrids (L233-A4, L231-A4, L120-A2 and L126-A3), three parents (P19A, Pa13 and IMC67) and two control clones (C151-61 and SCA6) as both callogenic and embryogenic. Petals proved to be the best embryogenic explant. PCG1 medium was both callogenic and embryogenic. The improved characterization by combination of factors from primary descriptors generated three secondary descriptors. This combination of factors allowed refining the characterization by identifying not only the best explant but also the best medium for expression of each genotype. The approach used here allows an accurate characterization of genotype and might be helpful in the regeneration in plantlets of superior genotypes of cocoa.

Key words: Cocoa tree, refined characterization, factors combination, descriptors, callogenic and embryogenic explants

INTRODUCTION

Theobroma cacao L., is a perennial, allogamous and diploïd (2n = 20) plant, native of rainforests of Amazon basin and others tropical areas of South and Central America (Wood and Lass, 1985). It takes on great economic importance. Average yields in merchant cocoa of the adult cocoa trees fields, constituted of non selected material, in the order of 250-500 kg ha^{-1} are relatively weak (Mossu, 1990; Braudeau, 1991; Guiltinan and Maximova, 2000). This is due not only to the high level of heterozygosity of cocoa tree leading to the generation of progenies with very heterogenous characteristics, but specially to the existence in these fields of the unselected genotypes from ungraded seeds (Braudeau, 1991; Guiltinan and Maximova, 2000; Maximova *et al*., 2002).

One of the means able to increase these yields would be the diffusion by the producers of selected superior genotypes propagated vegetatively (Mossu, 1990; Braudeau, 1991; Li *et al*., 1998). However, the methods of classic vegetative propagation which are rooted cuttings and grafting used to clone the superior plant material revealed some limits in their performances (Bertrand and Dupois, 1992).

To palliate these insufficiencies, the technique of micropropagation by somatic embryogenesis has been used as complementary solution to the classic vegetative propagation methods. The first works on the somatic embryogenesis have been reported by Esan (1975). Subsequent works allowed obtaining of primary somatic embryos from different tissues (Adu-Ampomah *et al*., 1988; Li *et al*., 1998; Tan and Furtek, 2003) and improvement of efficiency of their production (Alemanno, 1995; Li *et al*., 1998; Tan and Furtek, 2003). Others works improved maturation (Kononowicz and Janick, 1984; Alemanno, 1995; Li *et al*., 1998), germination of primary somatic embryos (Wang and Janick, 1984;

Corresponding Author: Auguste Emmanuel Issali, Centre National de Recherche Agronomique, 01 BP 1740 Abidjan 01, Côte d'Ivoire Tel: (225) 05 8217 28

Duhem *et al*., 1989; Alemanno, 1995; Li *et al*., 1998). In the same way, others works improved their conversion into plantlets with of rates varying between 30-80% (Adu-Ampomah *et al*., 1988; Wen and Kinsella, 1991; Figueira and Janick, 1993; Lopez-Baez *et al*., 1993; Li *et al*., 1998; Tan and Furtek, 2003). However, the production of somatic embryos remained, subject to some constraints. It depends on external factors as seasonal and temperature variations, rainfall, sunstroke, etc. (Esan, 1975; Tan and Furtek, 2003) and intrinsic one as plant physiology, genotype, etc. (Adu-Ampomah *et al*., 1988; Söndahl *et al*., 1993; Li *et al*., 1998; Maximova *et al*., 2002; Tan and Furtek, 2003). In cocoa tree, some performed works allowed the characterization of the genotypes (Adu-Ampomah *et al*., 1988; Alemanno, 1995; Li *et al*., 1998; Tan and Furtek, 2003), explant effect (Adu-Ampomah *et al*., 1988; Chatelet *et al*., 1992; Söndahl *et al*., 1993; Li *et al*., 1998; Tan and Furtek, 2003) and culture medium effect (Chatelet *et al*., 1992; Li *et al*., 1998; Tan and Furtek, 2003). This characterization was conducted with not more than two descriptors, studying separately the factors (genotype, explant and callus induction media). Yet the responses obtained on culture media result in synergy, inhibition and/or competition of several factors in presence (Heller *et al*., 1993). Therefore, the *in vitro* characterization used up to here may not be complete and could constitute a handicap in the knowledge of callogenic and embryogenic behaviour of studied genotypes. Thus an accurate characterization of genotype requires, combining of the factors. This study describes an improved characterization of the callogenic and embryogenic abilities of some promising genotypes using a combination of factors.

MATERIALS AND METHODS

Plant material: Plant materials were obtained from CNRA (Centre National de Recherche Agronomique) experimental plots of Bingerville (Côte d'Ivoire) from September 2002 to December 2004. Thirteen genotypes belonging to three groups were used: 1) a group composed of five parents including two males (IMC67, Pa150) and three females (P19A, Pa13 and Pa121); 2) a group constituted of six hybrid genotypes (L120-A2, L126-A3, L231-A4, L232-A9, L233-A4 and L330-A9) selected among the descendants of the group of parents and 3) a group of control genotypes constituted of two clones (SCA6 and C151-61) (Table 1).

Culture conditions and media: Measuring 4 to 5 mm of length, the flower buds were collected once per week, early in the morning and used as source of explants. Primary somatic embryos were produced as described by Li *et al*. (1998) by culturing staminode and petal explants on Primary Callus Growth (PCG) medium, followed by transfers to Secondary Callus Growth (SCG) medium and were maintained on plant growth regulator free Embryo

Table 1: Summary on the origin and the characteristics of each genotype

Genotypes		Origin	Characteristics
Parents	IMC67	Collected by Pound in Up Amazonia.	One of the ten better parents; very tolerant to witches' broom disease; good yield; vigorous.
	P19A	Clonal materiel of Up Amazonia origin collected by Pound.	Precocious and vigorous; mean yield, increases when associated with Amelonado or Trinitario.
	Pa13	Clonal materiel of Up Amazonia origin.	Precocious and vigorous; mean yield, increases when associated with Amelonado or Trinitario.
	Pa121	Clonal materiel of Up Amazonia origin.	Precocious and vigorous; mean yield, increases when associated with Amelonado or Trinitario.
	Pa150	Collected by Pound in Up Amazonia.	One of the ten best parents; precocious and vigorous; good yield; resisting at the pod rot (black pod) disease.
Hybrids	L120-A2	crossing descendent hybrid Pa13 x IMC67	Half sib of L232-A9, L126-A3 and L231-A4. Precocious and vigorous. Good shape and size of pods; good yield; good rate of fat matter
	L126-A3	Crossing descendent hybrid Pa121 x IMC67	Full sib of L231-A4, half sib L233-A4 and L120-A2. Precocious and vigorous. Good shape and size of pods; good yield; good rate of fat matter.
	L231-A4	Hybrid descended of the crossing Pa121 x IMC67	Full sib of L126-A3, half sib of L233-A4 and L120-A2. Precocious and vigorous. Good shape and size of pods; good yield; good rate of fat matter.
	L232-A9	Crossing descendent hybrid Pa13 x Pa150	Half sib of L120-A2 and L330-A9. Precocious and vigorous. Good shape and size of pods; good yield; good rate of fat matter.
	L233-A4	Crossing descendent hybrid Pa121 x Pa150	Half sib of L231-A4, L126-A3, L330-A9 and L232-A9. Precocious and vigorous. Good shape and size of pods; good yield; good rate of fat matter.
	L330-A9	Crossing descendent hybrid P19A x Pa150	Half sib of L233-A4 and L232-A9. Precocious and vigorous. Good shape and size of pods; good yield; good rate of fat matter.
Control clones	C151-61	Clonal material come from Venezuela	Very elevated fuitset rate. More sensitive to pod rot, to Mirideses and to malformations of pods due to Wilt.
	SCAVINA 6 (SCA 6)	Collected by Pound in upper Amazon close to the Sabina hacienda (Ecuador).	One of the ten best parents; very tolerant to witches' broom disease, resistant to Phytophthora, pod rot, but produces tiny beans; good yield; vigorous.

The following characteristics of studied genotypes were described by Lockwood and Gyamfi, 1979, Ngoran, 1996. All parents (IMC67, Pa121, Pa13, P19A, SCA6) of the hybrids were Upper Amazonian

Table 2: Growth regulator hormonal balances contained in the culture media used

Culture media		Hormonal balances
PCG	PCG3	[2,4 D]/[TDZ] : 4.52 µM/11.35 nM
	PCG1	[2,4 D]/[TDZ] : 9.04 µM/22.70 nM
	PCG4	[2,4 D]/[TDZ] : 18.08 µM/45.40 nM
SCG		[2,4 D]/[Kinetin] : 9.04 µM/1.394 µM
ED		Hormone free

PCG3 medium hormonal concentration was the weakest. PCG1 medium hormonal. concentration contained two-fold the one from PCG3 medium. In the same way, PCG4 medium hormonal concentration was four-fold the one from PCG3 medium

Development (ED) medium. Adaptations of the protocol concerned the hormonal concentrations of the of primary callus growth media (Table 2). Seven flower buds were used by Petri dish in all experimentations.

Characterization was carried out both in hybrids and in parents from three-factor factorial experiment (genotype, explant and callus induction medium). In hybrids, such a factorial design was organized by the following way : for each genotype (six hybrids and two control clones), two explants (staminodes and petals) were extracted from the flower buds and sowed in bulk on three distinct callus induction media (PCG1, PCG3 and PCG4).

This operation was triplicated for each genotype once a week. Furthermore, resemblances between hybrids and parents were researched. To this end, comparison of their general abilities (Table 5) and the average performances was used. General abilities were defined from average and coefficient of variation combination. Average performances of the hybrids were assessed first in comparison to those of the two control clones on three primary callus growth media (PCG1, PCG3 and PCG4) and second, to those of the five parents on two calli induction media (PCG1 and PCG3). These comparisons were performed among individuals belonging to the same group.

Variables measurement: At the end of every culture cycle of three months, five following variables were measured for each genotype : callogenic explants number, embryogenic explants number, embryos number per embryogenic explant, average number of embryos per embryogenic explant and percentage of embryogenesis (Table 3).

Statistical analysis of data: The raw data collected on the genotypes during the two years of study were transformed with the EXCEL software (Table 3). Most statistical tests were carried out at 5% level. In order to improve the genotypes characterization of cocoa tree, two types of descriptors were used: the primary and secondary descriptors. Primary and secondary denominations were arbitrary used to distinguish the

Table 3: Measured variables (primary descriptors) for the study of the callogenic and embryogenic abilities of the 13 genotypes used

Measured raw variables	Transformation used	Symbol of the variable
Callogenic explants number	Square root	NEXCAL
Embryogenic explants number	Square root	NEXEMB
Embryos number produced by embryogenic explant	Square root	NEMB
Average number of embryos per embryogenic explant	Square root	MEXEMB
Percentage of embryogenesis	Angular (sine arc of the percentage square root)	PE

Square root and angular transformations were performed to normalize the distributions

descriptors. The primary descriptors come from measured variables of the callogenesis and the somatic embryogenesis at the end of every culture cycle. Thus, for the characterization of factors constituted of more than two variants (genotype, callus induction medium, genotype-explant and genotype-induction medium), averages separation was performed by Dunnett T3 test (SPSS 10.1.3 software) and Student-Fisher LSD test (Xlstat 7.5.3 software). However, for the factor composed of two variants (explant), the averages were compared by contrasts method using Student-Fisher LSD test (SPSS). Characterization by combination of factors of the genotypes has generated three secondary descriptors: The general abilities, the favourable explants and the PCG media. The general abilities come from the combination of the callogenic and/or embryogenic average performances of the genotypes with stability (CV<30%) whereby a given genotype developed calli and/or somatic embryos. The favourable explants were identified with the averages comparison of variants of the combination of factors genotype and explant. In the same way, the favourable PCG media was identified with the averages separation of the variants of combination of factors genotype and callus induction media. These separations of averages allowed identifying of the explant and the PCG medium which show a better expression of a given genotype. In the same way, in order to separate coefficients of variation, 30 % level was used. The Principal Component Analysis (PCA) was used to identify the most relevant primary descriptors (Xlstat).

RESULTS

Identification of the most relevant primary descriptors: Principal Component Analysis allowed structuring the primary descriptors (Fig. 1). Basing on their contribution in percentage to construction of factorial axis, the callogenic explants number (Contribution-NEXCAL/F2 Axis = 98.12%) and the embryogenic explants number (Contribution-NEXEMB / F1 Axis = 27%), were identified as the most relevant descriptors.

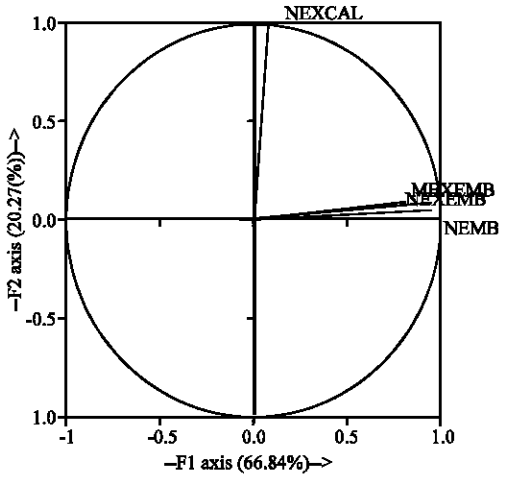

Fig. 1: Structuring of the 5 descriptors used on 1-2 plan of Principal Component Analysis (PCA) from correlation circle The 1-2 plan explained 87.11% of total variability (Fig. 1). F1 axis that described the somatic embryogenesis explained 66.84% of the total variability from initial data, while F2 axis describing the callogenesis, explained 20.27% of the residual variability unexplained by F1 axis. Global structuring of the descriptors produced two groups of which the first was constituted by the single descriptor of the callogenesis, the callogenic explants number (NEXCAL). The second group was formed by the four descriptors of the somatic embryogenesis: the embryogenic explants number (NEXEMB), the embryos number per embryogenic explant (NEMB), the average number of embryos per embryogenic explant (MEXEMB) and the percentage of embryogenesis (PE)

Characterization of callogenic and embryogenic abilities using separately the factors: For the callogenesis, the averages separation of the variants of the genotype factor generated four distinct groups, but stable (CV<30 %): The weakly (L232-A9), fairly (L233-A4), strongly (L231-A4, L120-A2, L330-A9, L126-A3, Pa150, P19A, Pa13, C151-61 and SCA6) and very strongly (Pa121 and IMC67) callogenic genotypes. Hybrid L232-A9 (NEXCAL = 2.062) was found to be the weakest callogenic, on the other hand IMC67 parent was the most callogenic (NEXCAL = 4.771). In the same way, three distinct effects but also stable (CV<30%) were observed with the primary callus growth: The media with high and weak hormonal concentration respectively PCG4 and PCG3 led to a weak callogenesis, while the medium at median hormonal concentration (PCG1) promoted a good callogenesis (Table 4).

Table 4: Classifications of the hybrids, the parents, the explant and the callus induction medium effect as a function of their callogenic and embryogenic abilities

Factors	Callogenesis (Nexcal)			Embryogenesis (Nexemb)		
Genotypes		Average	CV (%)		Average	CV (%)
Parents	Pa150	3.981c	3.06	Pa121	0.000a	a
	P19A	4.093c	2.78	Pa150	0.470b	54.40a
	Pa13	4.188c	2.55	IMC67	1.015b	24.14b
	Pa121	4.409d	2.69	P19A	1.940c	12.10b
	IMC67	4.771d	2.47	Pa13	2.600c	8.65b
Hybrids	L232-A9	2.062a	3.15	L232-A9	0.005a	1193.76a
	L233-A4	3.240b	2.01	L330-A9	0.090b	70.54a
	L231-A4	3.416c	1.87	L233-A4	0.100b	63.66a
	L120-A2	3.424c	1.87	L126-A3	0.220b	27.30b
	L330-A9	3.663c	1.77	L231-A4	0.235b	27.70b
	L126-A3	3.667c	1.75	L120-A2	0.260b	23.28b
Clones	C151-61	3.603c	1.75	C151-61	2.230c	2.69b
	SCA6	3.618c	1.77	SCA6	2.305c	2.60b
EXPLANTS	Pt	3.318	0.96	St	0.121a	27.12
	St	3.355	0.95	Pt	1.375b	2.40
MEDIA	PCG4	3.223a	1.21	PCG4	0.66a	6.67
	PCG3	3.339b	1.17	PCG3	0.77ab	5.71
	PCG1	3.448c	1.13	PCG1	0.814b	5.41

Classifications were carried out from transformed data. NEXCAL, Callogenic explants number; NEXEMB, Embryogenic explants number; Average, Values followed by different letter(s) (a, b, c or d) in same column are significantly different (DUNNETT T3 test at 5% level) for each factor. Reciprocally, values not followed of letters are not significantly different for a given factor; CV (%), coefficient of variation in percentage (significant for the superior averages at 30% level); CV: Unfollowed of letters (either a or b) are statistically identical for a given factor; Pa121 genotype recorded an infinite variation coefficient (CV>1 billion) relating to NEXEMB descriptor, it was materialized by an empty cell in table (drawee); Pt: Petals; St: Staminodes

Concerning somatic embryogenesis, three genotypes groups were identified : the weakly (L232-A9 and Pa121), fairly (L330-A9, L233-A4, L126-A3, L231-A4, L120-A2, Pa150 and IMC67) and strongly (C151-61, SCA6, P19A and Pa13) embryogenic genotypes. Among the genotypes constituting these groups, some appeared stable (CV<30%: L126-A3, L231-A4, L120-A2, C151-61, SCA6, IMC67, P19A and Pa13), while others appeared unstable (CV>30%: L232-A9, L330-A9, L233-A4, P121 and Pa150). Parent Pa121 (NEXEMB = 0.000) was recalcitrant, while Pa13 parent was the most embryogenic (NEXEMB = 2.600). Two distinct effects but stable (CV<30%) were identified for the factor explant: The staminodes expressed a weak embryogenic potential, compared to the one of the petals that appeared most embryogenic. In a stable way (CV<30%), the PCG4 medium did not promote a good embryogenesis in the difference of the PCG1 medium for all the hybrids studied (Table 4).

Characterization by combination of factors and analysis of resemblance between hybrids and parents: In order to improve the characterization of the callogenic and embryogenic abilities of the genotypes the combination of factors genotype-explant and genotype-induction medium allowed identifying favourable explants and PCG

Table 5: Characteristics of the hybrids and parents as a function of the explants and calli induction media by combined analysis of factors

Genotypes		Characteristics		
Parents	Pa121	General abilities	Very strong and stable	No
		Favourable PCG medium	PCG1	
		Favourable explants	Petals	
	Pa150	General abilities	Mean and stable	Mean but unstable
		Favourable PCG medium	PCG1	PCG1
		Favourable explants	Petals	Petals; staminodes
	IMC67	General abilities	Very strong and stable	Mean and stable
		Favourable PCG medium	PCG3	PCG3[a]
		Favourable explants	Staminodes	Petals[a]
	Pa13	General abilities	Mean and stable	Strong and stable
		Favourable PCG medium	PCG1	PCG3[a]
		Favourable explants	Staminodes	Petals[a]
	P19A	General abilities	Mean and stable	Strong and stable
		Favourable PCG medium	PCG1	PCG1[a]
		Favourable explants	Petals	Staminodes[a]
Hybrids	L232-A9	General abilities	Weak but stable	Bad and unstable
		Favourable PCG medium	PCG3	PCG4
		Favourable explants	Petals; staminodes	Staminodes; petals
	L330-A9	General abilities	Strong and stable	Mean but unstable
		Favourable PCG medium	PCG1[a]	PCG1
		Favourable explants	Staminodes; petals	Staminodes; petals
	L233-A4	General abilities	Mean and stable	Mean but unstable
		Favourable PCG medium	PCG1[a]	PCG1
		Favourable explants	Petals[a]	Petals[a]
	L231-A4	General abilities	Strong and stable	Mean and stable
		Favourable PCG medium	PCG3[a]	PCG4[a]
		Favourable explants	Staminodes[a]	Petals[a]
	L120-A2	General Abilities	Strong and stable	Mean and stable
		Favourable PCG medium	PCG1[a]	PCG4[a]
		Favourable explants	Staminodes[a]	Petals[a]
	L126-A3	General abilities	Strong and stable	Mean and stable
		Favourable PCG medium	PCG1[a]	PCG4[a]
		Favourable explants	Staminodes[a]	Petals[a]
Clones	C151-61	General abilities	Strong et stable	Strong and stable
		Favourable PCG medium	PCG1[a]	PCG3; PCG1
		Favourable explants	Petals[a]	Petals[a]
	SCA6	General abilities	Strong and stable	Strong and stable
		Favourable PCG medium	PCG3[a]	PCG1[a]
		Favourable explants	Petals, Staminodes	Petals[a]

Each genotype was characterized by three descriptors: general abilities, favourable explants and PCG medium. PCG medium and explants followed of an a ([a]): give the best and distinct answer. Pa121 genotype appeared refractory to the somatic embryogenesis, no favourable callus induction medium nor favourable explant exist for it. We materialized it by two empty cells in table (drawee)

media as secondary descriptors. Likewise the combination of average performances and stability (CV<30%) permitted identifying general abilities as secondary descriptors (Table 5).

The combination of factors genotype-explant, evidenced two genotypes (L233-A4 and C151-61) expressing their callogenic abilities through petals (Table 5). In addition, three genotypes (L213-A4, L120-A2 and L126-A3) were clearly highlighted showing their abilities through staminodes. On the other hand, eight genotypes (IMC67, Pa13, L233- A4, L231-A4, L120-A2, L126-A3, C151-61 and SCA6) expressed their embryogenic potential through petals. P19A parent was the sole genotype to display better embryogenic expression through staminodes (Table 5).

The combination of factors genotype-induction medium, evidenced five genotypes (L330-A9, L233-A4, L120-A2, L126-A3 and C151-61) showing better callogenic abilities on PCG1 medium. Additionally, two genotypes (L231-A4 and SCA6) preferentially expressed their callogenic abilities on PCG3. In the same way, two genotypes (L126-A3 and C151-61) were most callogenic on PCG1. Furthermore, three groups composed of two genotypes (P19A and SCA6; L231-A4 and L120-A2; IMC67 and Pa13) were found to express their embryogenic abilities, respectively on PCG1, PCG4 and PCG3 (Table 5).

Furthermore, the comparison of the callogenic general abilities of the hybrids to the one of the parents showed that for four crossings out of six, the hybrid presented a similar callogenic ability to the one of the male parent (Table 1, 5). The comparison of the callogenic average performances of the hybrids to those of the parents belonging to the same group previously identified as callogenic showed that those of two hybrids (L231-203 A4 and L120-A2) were both on this side of those of three parents (Pa150, P19A and Pa13) and the two control clones (SCA6 and C151-61) (Table 4 and Fig. 2).

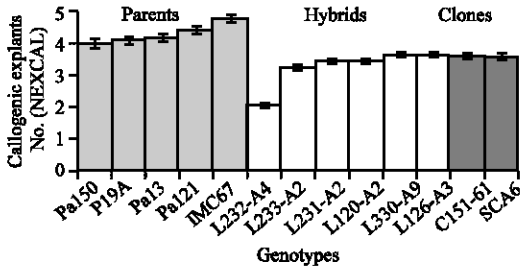

Fig. 2: Callogenic average performances from the hybrids and parents. Here, the raw data were transformed to normalize the distribution represented by the descriptor NEXCAL. For that purpose, square root transformation was used

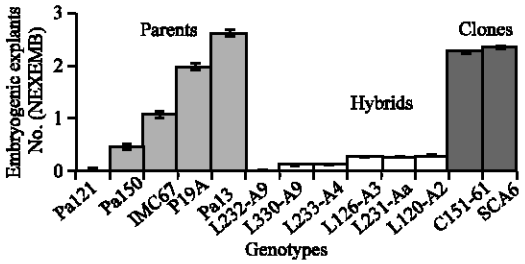

Fig. 3: Embryogenic average performances from the hybrids and parents. The raw data were transformed to normalize the distribution represented by the descriptor NEXEMB. For that purpose, square root transformation was used

The comparison of the embryogenic general abilities of the hybrids to those of the parents showed that for five crossings out of five, the hybrid presented a similar embryogenic ability to the one of the male parent (Table 1, 5). In the same way, the comparison of the embryogenic average performances of the hybrids to those of the parents belonging to the same embryogenic group showed that the ones of the hybrid (L232-A9) were similar to those of the parent (Pa121). However, those of five hybrids (L330-A9, L233-A4, L126-A3, L231-A4 and L120-A2) were both on this side of the ones of four parents (Pa150, IMC67, P19A and Pa13) and the two control clones (C151-61 and SCA6) (Table 4 and Fig. 3).

DISCUSSION

A method of improved characterization of the callogenic and embryogenic abilities of the genotypes of *Theobroma cacao* L., from two types of descriptors was used: the relevant primary descriptors (NEXCAL and NEXEMB) and the secondary ones (the general abilities, the Favourable explants and PCG media). To date, some works reported the individual characterization of the callogenic and embryogenic abilities of variants of some genotypes of cocoa tree (Adu-Ampomah *et al*., 1988; Alemanno, 1995; Li *et al*., 1998; Maximova *et al*., 2002; Tan and Furtek, 2003), of the effect of the explant variants extracted from immature seeds or flower buds (Adu-Ampomah *et al*., 1988; Alemanno, 1995; Li *et al*., 1998; Guilitinan and Maximova, 2000; Tan and Furtek, 2003) as well as of the effect of variants of the callus induction medium (Adu-Ampomah *et al*., 1988; Chatelet *et al*., 1992; Li *et al*., 1998; Tan and Furtek, 2003). However, no information is available on the improved characterization of the callogenic and embryogenic abilities of the genotypes and with greater reason those of *Theobroma cacao* L., from the secondary descriptors proposed here. The improved characterization procedure described has been applied to thirteen genotypes presenting various callogenic and embryogenic abilities. Furthermore, the comparison of the callogenic and embryogenic general abilities showed, strong resemblance between hybrids and male parents for nearly all of the crossings analyzed.

The separate characterization evidenced ten callogenic genotypes (L233-A4, L126-A3, L231-A4, L120-A2, Pa150, IMC67, C151-61, SCA6, P19A and Pa13), among them nine were embryogenic. In the same way, the petals were identified as most embryogenic, while this characterization did not allow identifying the most callogenic explant.

According to this method, all the genotypes expressed the embryogenic and callogenic abilities on PCG1 medium. Some studies reported similar characterization according to the callogenic and embryogenic potential (Adu-Ampomah *et al*., 1988; Alemanno, 1995; Maximova *et al*., 2002; Tan and Furtek, 2003). Callogenesis and somatic embryogenesis are subjected to variation depending on the genotype, explant and induction medium. The studied genotypes are more callogenic than embryogenic, as reported by Li *et al*. (1998). For the explants, petals were more embryogenic. However, many works (Alemanno, 1995; Li *et al*., 1998; Tan and Furtek, 2003; Antunez de Mayolo *et al*., 2003), indicated the superiority of the embryogenic staminodes potential compared to petals. In our case, it seems that the light temperature elevation allowing dissolving the myo-inositol vitamin would have genetically reprogrammed the petals nearer the zero point of the genetic program. This vitamin could influence the waking level during the dedifferentiation of the explants culturing, bringing the petals more nearer the zero point of the genetic program than the staminodes. According to Demarly and Sibi (1989), a genetic program nearer of embryonic state determines the embryogenic morphogenesis. The

myo-inositol is one of the constituents of the DKW vitamins. In short, the petals should be used in Côte d'Ivoire for the somatic embryos production. As response to this variation, we used a combination of factors as approach.

Contrary to the separate characterization, the characterization by combination genotype-explant allowed identifying the best callogenic and embryogenic explants. Additionally, the combination genotype-induction medium permitted the identification of the best callogenic and embryogenic media for each genotype (Table 5).

The resemblance observed between hybrids and their parents concerning the callogenic and embryogenic abilities, indicates the accuracy of the characterization method used. It suggests furthermore the existence of a possible genetic control of these two characters. But it is not excluded that these expressions are not from parents via paternal chromosomes, but rather from possible crossing-over between chromosomes during meiosis I. In this case, such an expression could come from the hybrid himself. However, the limited number of descendants and the absence of reciprocal crossings need some reservations in the conclusions to pull. To date, no study reported the heredity of the callogenic and embryogenic abilities. A selection program using the diallel-crossing systems would allow the understanding of this heredity.

It emerges that the characterization by combination of factors is more accurate. This could be helpful in the improvement of embryogenesis in genotypes with weak potentiality.

ACKNOWLEDGMENTS

We thank the Pennsylvania State University and the Biscuit Cake Chocolate and Sugar Confectionery Association (BCCCA) for financial support, M. Honoré G. OUATTARA and Professor Sévérin AKE for critical review of manuscript.

ABBREVIATIONS

2.4-dichlorophenoxyacetic acid; CV (%), Coefficient of variation in percentage; LSD, Least significant difference of Student Fisher; L_XA_Y (X Line, Y Tree), Hybrid under assessment, but not yet certified, non registered to the catalog, identified by its position on the experimental plot (x index) and by his rank on the line (y index), relating to the crossing which it is descended; PCG medium, Primary Callus Growth medium; TDZ, Thidiazuron - 1-phényl-3-(1, 2, 3 thiadiazol-5-yl) urea.

REFERENCES

Adu-Ampomah, Y., F.J. Novak, R. Afza, M. Van Duren and Perea-Dallos, 1988. Initiation and growth of somatic embryos of *Theobroma cacao* L. Café Cacao 32: 187-200.

Alemanno, L., 1995. Embryogenèse somatique du cacaoyer *Theobroma cacao* L.: Contraintes, progrès et perspectives. Spécialité: Physiologie végétale. Université Montpellier II. Sciences et Techniques du Languedoc. Thèse, pp: 193.

Antunez de, Mayolo, G., S.N. Maximova, S. Pishak and M.J. Guiltinan, 2003. Moxalactam as a counter-selection antibiotic for Agrobacterium mediated transformation and its positive effects on *Theobroma cacao* somatic embryogenesis. Plant Sci., 164: 607-615.

Bertrand, B. and V. Dupois, 1992. Contribution à la mise au point du clonage du cacaoyer (*Theobroma cacao* L.) par utilisation d'axes orthotropes. Contraintes et perspectives. Café Cacao, 36: 9-26.

Braudeau, J., 1991. Le cacaoyer. G.P. Paris (V). Maisonneuve et Larose, pp: 304.

Chatelet, P., N. Michaux-Ferrière and P. Dublin, 1992. Potentialités embryogènes du nucelle et du tégument interne de graines immatures de cacaoyer (*Theobroma cacao* L.). Histologie végétale/Plant histology. CR. Acad. Sci. Paris, t. 315, Série III, pp: 55-62.

Demarly, Y. and M. Sibi, 1989. Amélioration des Plantes et Biotechnologies. Universités francophones. UREF., John Libbey Eurotext, Paris, pp: 152.

Duhem, K., N. Le Mercier and P.H. Boxus, 1989. Données nouvelles sur l'induction et le développement d'embryons somatiques chez *Theobroma cacao* L. Café Cacao, 33: 9-14.

Esan, E.B., 1975. Tissue culture studies on cocoa (*Theobroma cacao* L.) - A supplementation of current research. In: Proceedings of the 5th International Conference on the Cacao Research, Ibadan, Nigeria, 1-9 Sept., pp: 116-125.

Figueira, A. and J. Janick, 1993. Development of nucellar somatic embryos of *Theobroma cacao* L. Acta Hortic., 336: 231-238.

Guiltinan, M.J. and S.N. Maximova, 2000. Recent advances in the tissue culture of Cocoa from somatic embryos to bentwood gardens. A short review. In: Proceedings of the International Workshop on New Technologies and Cocoa Breedings. Ingenic. 16-17th October 2000. Kota Kinabalu, Sabah, Malaysia, pp: 157-162.

Heller, R, R. Esnault and C.Lance, 1993. Nutrition. Tome 1. Physiologie végétale. 5th Edn. Masson. Paris-Milan-Barcelone, pp: 294.

Kononowicz, A.K. and J. Janick, 1984. *In vitro* development of zygotic embryos of *Theobroma cacao* L. J. Am. Soc. Hortic. Sci., 109: 266-269.

Li, Z., A. Traoré, S. Maximova and M.J. Guiltinan, 1998. Somatic embryogenesis and plant regeneration from floral explants of cocoa (*Theobroma cacao* L.) using Thidiazuron. *In vitro* Cell. Dev. Biol. Plant, 34: 293-299.

Lockwood, G. and M.M.O. Gyamfi, 1979. The CRIG cocoa germplasm collection with notes on codes used in the breeding programme at Tafo and elsewhere. Cocoa Research Institute, GHANA. Tech. Bull., 10: 62.

Lopez-Baez, O., H. Bollon, A. Eskes and V. Petiard, 1993. Embryogenèse somatique de cacaoyer (*Theobroma cacao* L.) à partir des pièces florales. C.R. Acad. Sci. Paris. Sciences de la vie/Life Sci., 316: 579-584.

Maximova, S.N., L. Alemanno, A. Young, N. Ferrière, A. Traoré and M.J. Guiltinan, 2002. Efficiency, genotypic variability and cellular origin of primary and secondary somatic embryogenesis of *Theobroma cacao* L. *In vitro* Cell. Dev. Biol. Plant, 38: 252-259.

Mossu, G., 1990. Le cacaoyer. Editeurs Lienhart and Cie, Aubenas d'Ardèche, France. ACCT-CTCAR., pp: 159.

Ngoran, J.A.K., 1996. Cocoa breeding in Côte d'Ivoire. Paper presented at the International workshop on the utilisation of the genetic resources of the international cocoa genebank. Trinidad, 23-28 June, pp: 7.

Söndahl, M.R., S. Lui and C. Bellato, 1993. Cacao somatic embryogenesis. Acta. Hortic., 336: 245-248.

Tan, C.L. and D.B. Furtek, 2003. Development of an *in vitro* regeneration system for *Theobroma cacao* from mature tissues. Plant Sci., 164: 407-412.

Wang, Y.C. and J. Janick, 1984. Inducing precocious germination in asexual embryos of cacao. Hortic. Sci., 19: 839-841.

Wen, M.C. and J.E. Kinsella, 1991. Somatic and plantlet regeneration of *Theobroma cacao* L. Food Technol., 5: 119-137. In: Cryopreservation of immature embryos of *Theobroma cacao* L. Plant Cell Reports. Springer-Verlag, 10: 144-147.

Wood, G.A.R. and R.A. Lass, 1985. Cocoa. Tropical Agriculture Series. 4th Edn. Longman, pp: 620.