Typology of seven *Vitellaria paradoxa* genotypes in relation to their response to callogenesis

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ABSTRACT

To type seven genotypes of shea tree relatively to their response to callogenesis, individual and typological approaches of classification were used. Endocarp and mesocarp explants from DK1, DK2, DK3, DK4, DK5, DK6 and DK7 genotypes were tested. C1, C2, C3 and C4 culture media were sowed with these explants and conducted for ninety one days. From individual approach, DK4 and DK6 showed better fruit size indicating that it will be necessary a few fruits number from them to obtain lots of explants to culture. DK2, DK3, DK5, DK6 and DK7 genotypes simultaneous displayed better weight of callogenic explants, callogenic explants number and percentage of positive response. These five genotypes might be used to search for culture media able to produce later somatic embryos. Mesocarp explants as well as C1 and C4 culture media were better responsive, postulating that they might be improved in future to produce somatic embryos. Typological approach, in conclusion, allowed the selecting of DK3, DK6 and DK7 genotypes expressing better compromise among the callogenic explant number, weight of callogenic explants and ratio of polar diameter out of equatorial diameter. They will be used in future to produce somatic embryos.
Keywords: mesocarp explant, endocarp explant, Côte d'Ivoire.

1. INTRODUCTION

Vitellaria paradoxa, is an agro-forestry species belonging to the Sapotaceae (http://en.wikipedia.org/wiki/Vitellaria paradoxa, on January 26 th 2013). Its natural range extends from eastern Senegal to the high plateau of Uganda. It plays an important role in economy of the countries which originated from sudanese savannas. The tree's main product is a fat (shea butter) extracted from the nuts. It is one of the rare local sources of vegetable fat in the region [1]. Shea butter is sold at about CFA Francs 400 per kg in the Bamako market (M.M. Sidibé, unpublished results). [2] estimated that 650,000 t shea nuts were collected throughout Africa in 2000. Additionally to local uses, shea butter is exported as smoked kernels for use in chocolate products and the pharmaceutical industry in European and other markets. It constitutes an important source of incomes for many women. Nevertheless, the strong fluctuations in availability of raw material limit the profit that these women can draw.

Shea tree is not cultivated. It mainly and naturally grows in fallows of cultivated soils with other crops. However, its growth very low creates heavy maintenance charges. In a similar manner, its fruit production is not precocious. Sure enough, it flowers about seven or eight years after planting. Likewise, its dispersion way which is essentially natural or wild confers it the status of picking crop.

Moreover, shea tree populations in natural parklands are declining [3] [4]. The drought, pressure of human populations, shortened fallows, agricultural mechanisation, pests' action such as mistletoe are the cause of such a declining. In the same way, the growth slowness also explains this declining [5]. One of the means to increase these populations is the artificial parklands regeneration with improved material plant.

Natural vegetative propagation seems null, although some authors have reported some possibilities of stooling [6]. Nonetheless, the speed of artificial regeneration of shea tree using the ancient biotechnologies such as grafting and layering is low. Indeed, the success ratio of grafting only reaches 25%, with the proviso that the grafts are not water stressed and that latex does not block the graft stock union [5]. In the same way, layering has also been a little successful, yet it is difficult [7]. Regarding rooted cuttings, no rooting was produced [6].

As an alternative, modern biotechnology using micropropagation via somatic embryogenesis has just performed [8] [9]. The former have produced somatic embryos using leafy explants, while the latter have made it via cotyledon explants. Nonetheless, some works such as those reported here were carried out before both above mentioned, namely in 2001. Unfortunately, they were not been published. In view of differences in used methodology between the works reported in present paper and both aforementioned, we have judged interesting to publish them. At the time of facts, we thought that it might exist plant tissue from shea tree able to proliferate onto at least one culture medium. The identifying of this tissue and culture medium might allow the mass-propagation of shea tree.

Our works aimed to test response to callogenesis of seven genotypes of shea tree for further somatic embryogenesis purposes using typological analyses.

2. MATERIALS AND METHODS

Plant material and experimental site
Seven genotypes, growing to the Kamonon Diabate's parkland of the station from CNRA (Centre National de Recherche Agronomique) in Côte d'Ivoire, were used. These are DK1, DK2, DK3, DK4, DK5, DK6 and DK7. They were about 20 m apart from one another. The Kamonon Diabate's parkland is located to the north of Côte d'Ivoire. The immature fruits harvested in this parkland were conveyed to Abidjan situated to the south of Côte d'Ivoire. The experiments concerning tissue culture were conducted from 2001 to 2002 at the Central Biotechnology Laboratory from CNRA based on Adiopodoumé site.

Culture media explants preparation, culture conditions and experimental design
Four culture media termed C1, C2, C3 and C4 were used during the experiments. The composition of these four media was reported (Table 1). Some of them were alike with respect to some supplements such as carbon source (glucose), plant growth regulators (2,4-D, TDZ), L-cystein, Thiamin-HCl, pH, gelling agent (Phytagel). Hence, C1 and C3 media were alike, because both proceeded from basal salt from Woody Plant Medium (Sigma M-6774). These four media differed from one another by some micronutrient basal salts and vitamins. Thus C2 medium is Primary Callus Growth (PCG) from [10]. It is used for cocoa tissue culture. C4 medium was constituted of Murashige and Skoog basal salts (Sigma M-5519) supplemented with additives shown in table 1.

Immature fruits were used as an explants source. The polar and equatorial diameters of these fruits were prior measured before their disinfection. The latter was done in two steps. Thus, in the first time, immature fruits were plunged in solution containing 900 ml water, 10 ml Heliopol liquid soap and 200 ml bleach at 8% for 20 min. At the end of such an operation, fruits were cleaned using polisher then rinsed thrice with distilled water. In the second phase, they were placed in third-filled bottles under orbital agitator containing a solution of calcium hypochlorid (Sigma-Aldrich) at 3% for 20 min too. Fruits were rinsed with distilled water, placed under laminar flow hood for 20 min then dissected later on. The autoclaving of media was done for 20 min.

The explants about 0.6 cm side were put in Petri dishes containing previously described media. The experiment was planned according to 2 x 7 x 4 factorial scheme in a completely randomised design. Treatment consisted of explants (2 in all) deriving from a genotype (7 in all) placed in a culture medium (4 in all). Each treatment being triplicated, in all, hundred sixty eight treatments were obtained at the end of each culture initiation. The initiated cultures were conducted in the dark at 24°C. After fourteen days, callogenic explants were subcultured onto the same medium, but hormone-free. The callogenic explants were then subcultured thrice every 21-day. In all, ninety one days were necessary to initiate and conduct the cultures.
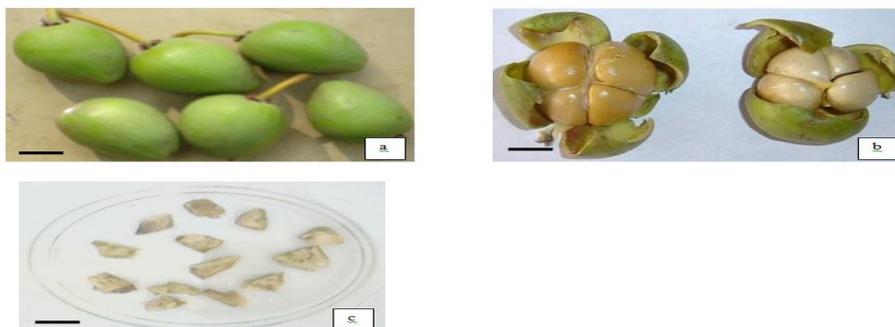

Figure 1: Immature entire (a) and mature (b) fruits as well as culture medium sowed with mesocarp of immature fruits (c). Bar for pictures "a" and "b" = 1 mm; for picture "c" = 1.44 mm.

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Table 1: Composition of four culture media termed C1, C2, C3 and C4 for 1 litre.

Medium	Composition	Quantity	Medium	Composition	Quantity
C1	Mc Cown's salt	0.6 g	**C3**	Mc Cown's salt	2.3 g
	Dioscorea micronutrients	20 ml		DKW vitamins	1 ml
	DKW vitamins	1 ml		Amino-acids (1000x)	1 ml
	Amino-acids (1000x)	1 ml		Glucose	20 g
	Dioscorea vitamins	1 ml		2,4-D (1mg/ml)	1 ml
	Glucose	20 g		TDZ (1mg/ml)	2 ml
	2,4-D (1mg/ml)	1 ml		L-cystein	0.2 g
	TDZ (1mg/ml)	2 ml		Thiamin-HCl	0.1 g
	L-cystein	0.2 g		pH	5.7
	Thiamin-HCl	0.1g		Phytagel	2.2 g
	pH	5.7	**C4**	MS	4.43g
	Phytagel	2.2 g		Glucose	20 g
C2	DKW macro A (10x)	100 ml		2,4-D (1mg/ml)	1 ml
	DKW macro B (10x)	100 ml		TDZ (1mg/ml)	2 ml
	DKW micro (100x)	10 ml		L-cystein	0.2 g
	DKW vitamins	1 ml		Thiamin-HCl	0.1 g
	Amino-acids	1 ml		pH	5.7
	Glucose	20 g		Phytagel	2.2 g
	2,4-D (1mg/ml)	1 ml			
	TDZ (1mg/ml)	2 ml			
	L-cystein	0.2 g			
	Thiamin-HCl	0.1 g			
	pH	5.7			
	Phytagel	2.2 g			

Measurements
Concerning the descriptors of fruits size, before the culture initiation, polar and equatorial diameters (Poldiam and Eqdiam) were measured, while ratio of polar diameter out of equatorial diameter (Poleq) was calculated. With respect to tissue culture, explants were weighed before the culture initiation as well as at each subculture (Weight). At the end of each culture initiation, the callogenic explants number (Ncal) was scored. The percentage of positive response (Respos) was calculated.

Data analysis
Collected data were processed by Analysis of variance (ANOVA), Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) with Xlstat 2007.6 and SPSS 12.0 softwares. The ANOVA was run to separate the averages according to Newman-Keuls' and Z Student's tests at 5% threshold. To normalise the measured distributions and equalise variance, the polar diameter and ratio of diameter polar out of equatorial diameter as well as callogenic explants number were transformed to $\sqrt{x}$, whereas percentage of positive response was it to arcsin$\sqrt{y}$. Moreover, the weight evolution of callogenic explants as a function of time was analysed. The PCA was performed to structure the descriptors and individuals represented by genotypes. The choice of factorial axes and relevant descriptors was done using Kaiser and angle as well as representation quality criteria, respectively. Kaiser's criterion would like that the factorial axes whose eigenvalues are higher than 1, are retained. Angle criterion is based on the existence of an angle on the scree plot corresponding to brutal fall of the eigenvalue. This brutal fall takes place at inflection point. Relevant descriptors were chosen from their representation quality termed QLT_{kl} (QLT_{kl} of a descriptor $= \cos^2$ axis1 + $\cos^2$ axis2) at 0.9 threshold and Pearson's linear correlation coefficient. The most relevant descriptors chosen from the PCA were used to type the genotypes by means of the HCA.

3. RESULTS AND DISCUSSION

Effect of the genotype on the expression of fruits size using analysis by descriptor
For the equatorial diameter, three statistically different groups of genotypes were noted. First, constituted of DK3 and DK7 genotypes, was characterised by weak equatorial diameter. Second, composed of DK1 genotype, stood out from two others by mean equatorial diameter. Third, consisting of DK2, DK4, DK5 and DK6 genotype, was distinguishable by strong equatorial diameter (Table 2).
Regarding the polar diameter, two significantly different groups of genotypes were identified: 1) group constituted of DK1, DK2, DK3, DK5 and DK7 genotypes; it differed from the other by low polar diameter and 2) group comprising DK4 and DK6 genotypes, was characterized by high equatorial diameter (Table 2).

As regards the ratio of polar diameter out of equatorial diameter, two homogeneous groups were observed. First, composed of DK2, DK5 and DK1 genotypes, was marked by low ratio of polar diameter out of equatorial diameter. Second, constituted of DK3, DK4, DK6 and DK7 genotypes, stood out from the former by high ratio of polar diameter out of equatorial diameter. The obtained ratios ranged from 1.04 to 1.7 suggesting that the shape of measured fruits is oblong (Figure 1). Variability around average varied from 1.34 to 3.13 (Table 2).

In brief, DK4 and DK6 recorded the highest fruits size. In shea tree, oblong shape is frequently met, but the round one also exists [11]. The best expression of the fruit size of these two genotypes might be related to environment or genes effect. For these two genotypes, it will be necessary to use a few fruits to obtain high number of explants for tissue culture.

Table 2: Averages classification of the equatorial and polar diameters and their ratio

Dependent descriptor	Genotype	Transformed average	CV (%)	Untransformed average
Eqdiam	DK3	1.867a	3.00	-
	DK7	1.944a	2.16	-
	DK1	2.100b	2.67	-
	DK6	2.288c	1.84	-
	DK2	2.322cd	2.41	-
	DK4	2.367cd	2.37	-
	DK5	2.456d	1.71	-
Poldiam	DK1	1.490a	2.08	2.220
	DK3	1.505a	2.06	2.265
	DK2	1.557a	1.99	2.424
	DK5	1.584a	1.45	2.509
	DK7	1.586a	1.45	2.515
	DK4	1.693b	1.83	2.866
	DK6	1.711b	1.34	2.928
Poleq	DK5	1.022a	2.74	1.044
	DK2	1.052a	3.61	1.107
	DK1	1.058a	3.59	1.119
	DK4	1.214b	3.13	1.474
	DK3	1.215b	3.13	1.476
	DK6	1.284b	2.18	1.649
	DK7	1.306b	2.14	1.706

Genotype, culture medium and explant effects on the expression of callogenic explants weight and number as well as percentage of positive response

In figure 2, genotype, explant and medium effects were evidenced. Indeed, for genotype effect, onto C1, C2 and C3 media, the weight of DK5 was 0.795, 0.884, 0.653 g, respectively. It expressed there better weight growth than six other genotypes. This genotype effect was confirmed by means of averages separation (Table 3). In this one, concerning descriptor termed the weight of callogenic explants, two statistically different groups of genotypes were noted. The former represented by the only one DK4 genotype was characterised by low weight of callogenic explants. The latter, constituted of DK1, DK2, DK3, DK5, DK6 and DK7 genotypes, was distinguishable by high weight of callogenic explants.

This genotype effect was also illustrated through the callogenic explants number and percentage of positive response (Table 3). Sure enough, with respect to the callogenic explants number, three genotype groups were classified : 1) group composed of genotype DK4 was marked by weak production potential of calli; 2) group constituted of DK1 genotype stood out from two others by mean production potential of calli; 3) group consisting of DK2, DK3, DK5, DK6 and DK7 genotypes was characterised by strong production potential of calli.

For the percentage of positive response, three groups were observed. First, consisted of DK4 genotype, differed from others by low percentage of positive response. Second, comprising DK1 genotype, stood out from two others by mean percentage of positive response. Third, composed of DK2, DK3, DK5, DK6 and DK7, was characterised by high percentage of positive response (Table 3).

In figure 2, explants effect was displayed through difference of weight between callogenic mesocarp and endocarp explants. Sure enough the weight of callogenic endocarp explants varied from 0.052 to 0.183 g, whereas those from mesocarp oscillated from 0.100 to 0.884 g. This explant effect was also confirmed by averages comparison (Table 3). In this table, two statistically different groups were noted regarding the weight of callogenic explants, callogenic explants number and percentage of positive response. Callogenic explants from endocarp reduced callogenesis, while those from mesocarp favoured it.

In the same figure 2, calli induction medium effect was evidenced. Indeed, the media C1 and C4 favoured mesocarp callogenesis than C3 media. Sure enough, these two previously cited media recorded mesocarp weights 0.807 and 0,884 g as against 0.653 onto C3 medium. This was also confirmed from means comparison (Table 3). In the latter, with respect to the callogenic explants number and percentage of positive response, two distinct effects were identified. C1 and C4 induction media favoured callogenesis, while C2 and C3 reduced that.

In sum, DK2, DK3, DK5, DK6 and DK7 genotypes simultaneous expressed good weight of callogenic explants, callogenis explants number and percentage of positive response. It concerns the genotype effect. This one was evidenced in numerous plants in vitro cultured such as cocoa tree [10] [12] [13], soybean [14], sugar cane [15], rice [16] among others. It depends essentially on genes expression. Consequently, these five previously cited genotypes might be selected for searching for media able to produce later somatic embryos.

Mesocarp explants in vitro cultured expressed the highest callogenic effect. It is about explant effect which, as genotype effect, was displayed in several other plants such as cocoa [10] [17] [18], rice [16], sorghum [19], among others. From leafy explants of shea tree, [8] produced calli then somatic embryos from the latter. In contrast, [9] have just produced somatic embryos on cotyledon calli of shea tree. We succeeded in producing callogenic explants from immature fruits mesocarp. Although our works were late published, nonetheless they were carried out before both above mentioned, namely in 2001. Indeed, the first authors used leafy explants in 2008, while the second have worked on cotyledons in 2012. We have used mesocarp explants. Our works merited to be published. That is why, efforts must be pursued to explore also such a way.

In contrast, endocarp was not responsive. All of explants of this tissue did not proliferate onto four tested media. They died eight days after the initiation of culture. Cotyledon explants were not also responsive onto these media (data not shown). Nevertheless, leafy explants were responsive onto four tested culture media (data not shown).

C1 and C4 culture media revealed the best callogenic effects. That is culture medium effect. It was evidenced on numerous plants. C1 culture medium is constituted of Mc Cown basal salt. It is adapted to woody plants [10]. As for C4 culture medium, it is composed of Toshio Murashige and Folke Skoog basal salt [20]. It was created by aforementioned authors to culture tobacco tissue. This medium was adapted and diversified to respond to specific plants need. On shea tree, [8] and [9] used MS/2 and MS media, respectively, to produce both calli and somatic embryos. Hence, C1 and C4 culture media must be used to pursue the research for somatic embryos production. This, independently of protocol used in [8] and [9].
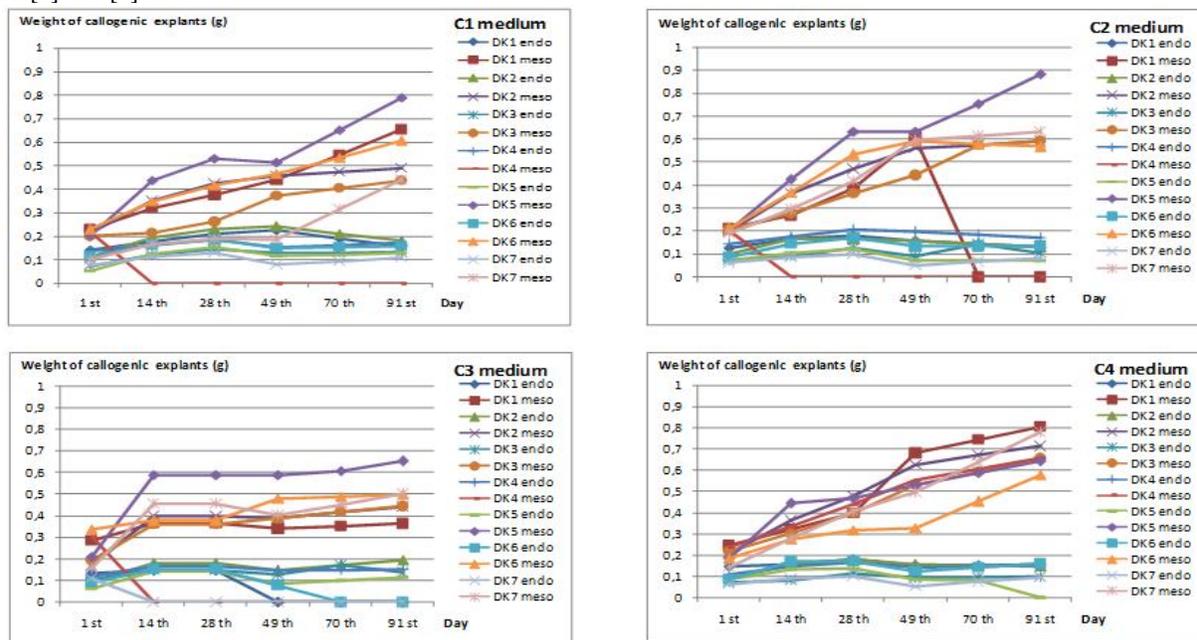

Figure 2 : Weight evolution of the callogenic explants of immature fruits of seven Shea tree genotypes onto four callus induction media.

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Table 3: Classification of the averages of weight, callogenic explants number and percentage of positive response as a function of genotype, callus induction media and explant nature.

Dependent descriptor	Genotype	Transformed average*	CV (%)*	Untransformed average*	Dependent descriptor	Explant*	Transformed Average*	CV(%)*	Untransformed average*
Weight	DK4	0.154a	10.39	-	Weight	endo	0.125a	7.20	-
	DK7	0.226b	7.08	-		meso	0.382b	2.36	-
	DK3	0.247bc	6.48	-	Ncal	endo	0.000a	∞	0.00
	DK1	0.264bc	6.06	-		meso	2.726b	11.01	7.43
	DK6	0.274bc	5.84	-	Respos	endo	0.000a	∞	0.00%
	DK2	0.297bc	5.39	-		meso	0.720b	6.97	43.18%
	DK5	0.312c	5.13	-					

Dependent descriptor	Genotype	Transformed average*	CV (%)*	Untransformed average*	Dependent descriptor	Induction medium	Transformed average*	CV(%)*	Untransformed average*
Ncal	DK4	0.331a	10.42	0.110	Weight	c3	0.222a	5.41	-
	DK1	0.770b	18.23	0.593		c1	0.244ab	4.92	-
	DK3	1.470c	13.61	2.161		c2	0.253ab	4.74	-
	DK5	1.616c	12.38	2.611		c4	0.294b	4.08	-
	DK7	1.721c	11.62	2.962	Ncal	c2	1.183a	1.69	1.399
	DK2	1.759c	1.71	3.094		c3	1.224a	1.63	1.498
	DK6	1.874c	10.67	3.512		c1	1.457b	1.37	2.123
Respos	DK4	0.079a	2.53	0.62%		c4	1.587b	1.26	2.519
	DK1	0.184b	16.30	3.35%	Respos	c2	0.299a	6.69	8.68
	DK3	0.361c	2.77	12.48%		c3	0.314a	6.37	9.54%
	DK5	0.405cd	2.22	15.53%		c1	0.396b	5.05	14.90%
	DK7	0.447cde	4.47	18.69%		c4	0.426b	4.69	17.10%
	DK2	0.497de	8.05	22.73%					
	DK6	0.535e	37.38	26.00%					

Legend : Transformed average* : The descriptor "weight" was not transformed. This explains the lack of numbers in corresponding column "Untransformed average". CV(%)* : That is coefficient of variation. It was calculated from the formula : (standard deviation / average) x 100. Untransformed average*: Ncal: It was transformed to square root of callogenic explant number. Respos : It was transformed to arc sine of the square root of positive response percentage. The inverse of each function was obtained from $(x)^2$ and $100 \times [\sin(y)]^2$ formulas. Explant*: For comparison involving two averages, Student's Z test was used.

Analysis with the descriptors as a whole
Descriptors relevance and typing of the shea genotypes
Taking into account both the square cosine of initial descriptors on the factorial axes as well as Pearson's linear correlation, the callogenic explants number, weight of callogenic explants, ratio of polar diameter out of equatorial diameter were retained for the rest of the study. However, the percentage of positive response, polar and equatorial diameter were eliminated from the study (Figures 3 and 4).
From these three descriptors, three individual groups were preliminarily observed on the principal plane 1-2. First G1 composed of DK1, DK2 and DK5 genotypes. Second G2 constituted of DK3, DK6 and DK7 genotypes. Third G3 consisting of sole DK4 genotype (Figure 6).
In brief, 1, 2 and 3 factorial axes and the callogenic explants number, weight of callogenic explants, ratio of polar diameter out of equatorial diameter allowed the structuring of genotypes used. The typological approach consisting in using Kaiser and angle criteria as well as 0.9 likelihood for representation quality and Pearson's linear correlation coefficient for choice of relevant descriptors was used in [21].

Footnote related to the PCA
The choice of factorial axes was based on two criteria. It concerns Kaiser and angle criteria. According to the Kaiser's criterion, the axes whose eigenvalues are greater than 1 must be retained for the rest of the study. Here, out of five factorial axes, sole three namely F1 axis (eigenvalue = 2.542), F2 axis (eigenvalue = 1.886) and F3 axis (eigenvalue = 1.507) were effectively retained. After the angle criterion, the fourth point having as coordinates (F4;3) was the point of inflection. So, the three points preceding this fourth was chosen. In sum, the first three factorial axes synthesised the information contained in six initial descriptors. They accounted for 98.91% total variation. Thus, the first, second and third factorial axes explained 41.53, 32.09 and 25.29% variation, respectively. They were chosen to interpret the variability on a level with seven genotypes studied (Figures 4 and 5).
The choice of descriptors to use was guided by their representation quality termed QLT_{kl}. The Ncal and Respos descriptors were well both represented on 1-2 and 1-3 planes (QLT_{kl}(1-2) = 0.994; QLT_{kl}(1-3) = 0.990). The Weight descriptor was salient on 1-2 plane (QLT_{kl}(1-2) = 0.963), but not on 1-3 plane (QLT_{kl}(1-3) = 0.490). The Eqdiam and Poldiam descriptors were not both prominent on 1-2 and 1-3 planes (QLT_{kl}(1-2) for Eqdiam = 0.104; QLT_{kl}(1-3) for Eqdiam = 0.893; QLT_{kl}(1-2) for Poldiam = 0.391; QLT_{kl}(1-3) for Poldiam = 0.608). In contrast, the Poleq descriptor was salient on 1-2 plane (QLT_{kl}(1-2) = 0.971), but not on the 1-3 one (QLT_{kl}(1-3) = 0.039). So, The Ncal, Respos,

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Poleq and Weight descriptors expressed representation quality higher than 0.9. Nonetheless, Ncal and Respos were significantly and positively correlated (r Ncal – Respos = +1*). Both had got the same representation quality. The percentage of positive response was eliminated from the study. In all, the Ncal, Weight and Poleq descriptors were retained and used for the rest of the study (Table 4).

The projection of individuals on the 1-2 and 1-3 planes accounting for 73.61 and 66.82% variation, respectively, allowed the clustering of genotypes. The 1-2 plane especially provided three morphological groups: 1) G1 composed of DK1, DK2 and DK5 genotypes, 2) G2 constituted of DK3, DK6 and DK7 genotypes and 3) G3 consisting of sole DK4 genotype. G2 and G3 groups differed from that of G1 on the 1 component. In the same way, G2 group stood out from G3 on the 2 component. On the three components, no clear structuring was noted (Figure 5).

Footnote relating to the HCA

Choosing three groups, the truncation was carried out about 6.9 genetic distance represented by euclidian distance. The three morphological entities identified from the PCA were confirmed by the HCA. The individuals from G1 group were characterised by high callogenic explants number, weight of callogenic explants, but low ratio of polar diameter out of equatorial diameter. G2 group individuals were distinguishable by high callogenic explants number, weight of callogenic explants and ratio of polar diameter out of equatorial diameter. Likewise, on the same component, G3 group was marked by low callogenic explants number and weight of callogenic explants, but high ratio of polar diameter out of equatorial diameter.

Moreover, further consideration of the genetic distance, expressed through euclidian distance from distances matrix, measured among three groups seemed to show that these ones are effectively distinct (Table 6).

Preliminarily identified groups from the PCA, on 1-2 plane, were confirmed by the HCA. G2 groups constituted of DK3, DK6 and DK7 genotypes better presented compromise with respect to callogenesis potential, calli weight and fruits size (Figure 7; Table 5).

Figure 3: Plot deriving from the Scree Plot Test allowing the determination of factorial axes number to use for the PCA.
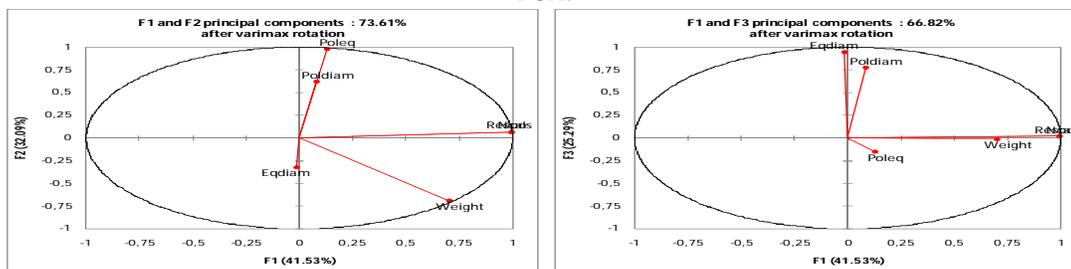

Figure 4: Representation quality on the (1-2) and (1-3) planes of used descriptors through correlation circles of the PCA.
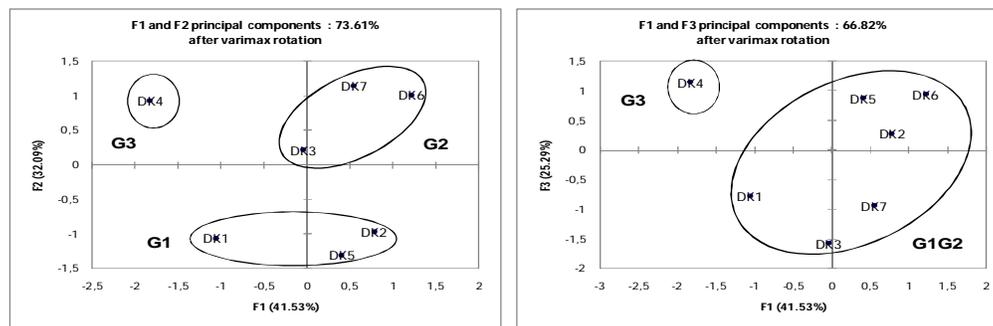

Figure 5: Structuring of the individuals represented by genotypes on the (1-2) and (1-3) planes through components maps of the PCA.

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Table 4: Square cosine of studied descriptors on the three factorial axes retained as well as their representation quality on the 1-2 and 1-3 planes.

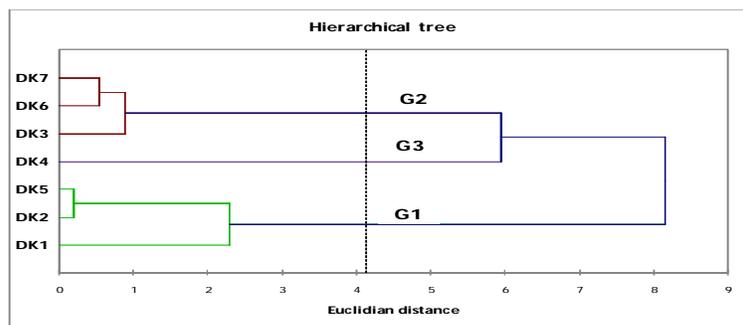| Descriptors	Axis			QLT$_{kl}$	
	F1	F2	F3	1-2	1-3
Ncal	0.989	0.004	0.000	0.994	0.990
Respos	0.989	0.004	0.000	0.994	0.990
Weight	0.490	0.473	0.000	0.963	0.490
Eqdiam	0.000	0.104	0.893	0.104	0.893
Poldiam	0.007	0.385	0.601	0.391	0.608
Poleq	0.016	0.955	0.023	0.971	0.039

Figure 6: Hierarchical tree deriving from the HCA clustering the individuals represented by genotypes.

Table 5: Averages of three relevant descriptors for each of identified groups from the HCA.

Classe	Ncal	Weight	Poleq
G1	4.500	0.291	1.044
G2	5.833	0.249	1.268
G3	0.875	0.154	1.214
Average	3.736	0.231	1.176

Table 6: Measured proximity among three identified groups through the euclidian distance of the distances matrix.

	G1	G2	G3
G1	0	2.073	2.814
G2	2.073	0	2.404
G3	2.814	2.404	0

In sum, with the descriptors as a whole DK3, DK6 and DK7 genotypes from G2 group expressed good compromise between callogenesis potential and fruits size. The typological classification was better than the individual one provided by averages comparison incorporated with the Anova [18]. Trees are about 20 m apart from one another on the Kamonon Diabate's parkland from CNRA. With such a gap, it can be believed that the three previously cited genotypes might be related. They might come from a same female tree. The resemblance among them, relatively to their response to callogenesis, suggests that they are very little different one another. Indeed, a low genetic diversity was evidenced in shea tree in spite of its cross-pollinated mating system (Table 6; [22] [23] [24]). So, finally DK3, DK6 and DK7 genotypes were selected for further somatic embryogenesis purposes.

4. CONCLUSION

At the time of facts we postulated the assumption that it might exist tissue from *Vitellaria paradoxa* able to proliferate onto at least one culture medium. This one would favour the mass-propagation of this forestry species. At the end of our works, one tissue and two culture media were selected for calli production. Indeed using classification by descriptor, DK4 and DK6 genotypes expressed the highest fruits size. Likewise, five genotypes, namely DK2, DK3, DK5, DK6 and DK7 showed better expression of weight of callogenic explants, callogenic explants number and percentage of

positive response. Nonetheless, resorting to typological approach, DK3, DK6 and DK7 genotypes displayed better compromise between callogenic potential and fruits size. They were selected for further somatic embryogenesis works. Mesocarp explants as well as C1 and C4 culture media showed better response to callogenesis expression. They will be later used to produce somatic embryos.

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