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Biometric Analysis on Genetic Divergence between Parental and Regenerated Accessions in Tall Coconut Palms (*Cocos nucifera* L.) from International Genebank for Africa and the Indian Ocean

Saraka Didier Martial Yao[1], Wentoin Alimata Marie Pierre Daramcoum[1]*,
Eric-Blanchard Zadjéhi Koffi[1], Konan Jean Louis Konan[2], Nafan Diarrassouba[1],
Bourdeix Roland[3], Raoul Sylvère Sie[4], Irié Arsène Zoro Bi[4]

[1]Department of Biochemistry and Genetics, Pedagogical and Research Unit (UPR) of Genetics, Shea Breeding Program, University of Peleforo Gon Coulibaly (UPGC), Korhogo, Côte d'Ivoire
[2]Centre National de Recherche Agronomique (CNRA), Marc Delorme Research Station of Port-Bouët, Abidjan, Côte d'Ivoire
[3]CEFE-CNRS, Montpellier, France
[4]Department of Natural Science, Nangui Abrogoua University, Abidjan, Côte d'Ivoire
Email: *mariepierrekiss@yahoo.fr

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Abstract

This study endeavour assesses agromorphological likeness between initial introductions and regenerated accessions at the International Coconut Genebank for Africa and the Indian Ocean (ICG-AIO) based in Côte d'Ivoire. Ten couples of parental (G0) and regenerated (G1) accessions of Tall coconut palms were analyzed using Principal Component Analysis (PCA) and Multiple Analysis of Variance (MANOVA) from 26 agromorphological characters. The main results showed a relative decrease in the expression of the phenotypical traits concerning the component of the fruit, height and vigor of the stem and yield of bunches and fruits after one regeneration cycle. But, a high proportion (69%) of studied characters from leaf, inflorescence and nut components showed likeness between G0 and G1 accessions. After one regeneration cycle, the controlled pollination method guarantees significant conservation of the expression of the majority of agromorphological traits. Consequently, regenerated accessions of Tall coconut palms can be used to pursue research and development programs in Côte d'Ivoire.

Keywords

Agromorphological Likeness, Controlled Pollination, Côte d'Ivoire, Regenerated Accessions, Tall Coconut

1. Introduction

Coconut palm (*Cocos nucifera* L.) is an oleaginous and perennial crop widely cultivated in tropical humid areas. Pacific and Indo-Atlantic oceanic basins are the two geographical origins of coconut palms proposed by Gunn [1]. Natural pollinations are realized essentially by insects and wind [2]. Coconut palms are generally classified into Tall and Dwarf populations from the Arecaceae family [3]. Dwarfs and Talls represent respectively about 5% and 95% of coconut populations [1]. Oil extracted from kernel provides fatty acid to the oil-chemical industry [4]. Coconut palm growth is indefinite until its dead. Trunk elongation is linked to apical meristem growth, age, climatic risks and coconut populations. Tall coconut palms grow more quickly than the Dwarfs [5].

In Côte d'Ivoire, more than 53 coconut populations have been introduced since 1952 to allow breeders to develop improved hybrids for supporting the coconut sector worldwide. The important height growth of the coconut palms really limits field management and crossing activities of the accessions. When the genetic resources are conserved in the field, the period of their exploitation for research and seed production is around 20 and 30 years respectively for the Tall and Dwarf coconut palms. After these periods, the heights of the coconut palms are more than 12 m and their inflorescences become inaccessible [6]. Thus, manipulations of inflorescence organs become dangerous. This constraint which appeared in 1988 limits using coconut genebank for research and development. Regeneration of the aged accessions appeared as the best solution for the curators. From Tall coconut palms, preferentially outcrossing, seed production for regeneration consists of crossing parental individuals using a controlled pollination technique [7]. Concerning the Dwarf palms which are a 95% of the self-pollinated rate, regeneration of the accessions doesn't require a controlled pollination method. The controlled pollination method includes risks of genetic erosion involving from the sampling of parental individuals. Indeed, selected batches of parental palms from the original coconut population can induce a loss of genes, especially in Tall palms which are more heterozygote [8] [9]. Therefore, the risks of gene losses and genetic divergence between parental and regenerated coconut accessions seem to be feared.

Now, directives about regenerations of plant collection recommend following up the accession identities through regeneration cycles [10]. Agromorphological descriptors are heritable, easy to assess and follow through the generations. Also, they constitute interest characters that guide breeders for coconut improvement. However, climatic change and modifications concerning soil chemical properties could influence the expression of the morphological traits through generations of coconut palm accessions. Consequently, current work aimed to assess agro-morphological divergence between parental and regenerated accessions of Tall coconut palms located at the international field genebank of Côte d'Ivoire.

2. Materials and Methods

2.1. Plant Material and Experimental Site

Ten couples of parental and regenerated Tall coconut accessions planted at 143 trees per hectare have been used (**Table 1**). Adult palms aged between 9 and 23 years from International Coconut Genebank for Africa and Indian Ocean (ICG-AIO) located in Marc Delorme research station were assessed. Genebank is located between 5˚14' and 5˚15' of North latitude and between 3˚54' and 3˚55' of West longitude.

The parental plant material (G0), initially introduced in ICG-AIO from 1968 to 1975 consisted of 10 tall coconut accessions planted in six collection plots (N˚081 planted in 1968, plot N˚091 planted in 1969, plot N˚101 planted in 1970, plot N˚102 planted in 1975, plot N˚111 planted in 1970 and plot N˚112 planted in 1972). The parental accessions constitute the reference population in the study and the ancestors of the regenerates. The accessions were introduced into Côte d'Ivoire as seed from five regions: Africa, South Pacific and Far East.

The regenerated plant material (G1) consisted of 10 regenerated accessions of Grand coconut. It was obtained by the controlled pollination method from parental accessions already present in Côte d'Ivoire and planted on 2 plots (plot N˚081 planted in 2002 and plot N˚091 planted in 1988) [11]. The crosses carried out within each of the parental accessions to obtain the regenerated ones are of type G0 × G0. The WAT accession was used as control in the trials at each parental (G0) and regenerated (G1) generation.

The climate is equatorial type with two humid and dry seasons (**Figure 1**). Monthly mean of rainfall distribution during decades that preceded field genebank regeneration and from 1988 to 2010 remains similar (**Figure 1**).

Table 1. Details of parental and regenerated Tall coconut accession studied including West African Tall (WAT) used as control in experimental plots.

		G0 generation			G1 generation		
No.	Accessions (Code)	No. Plot	Control	Year of planting	No. Plot	Control	Year of planting
1	Rennel Island Tall (RIT)	081	WAT	1968	091	WAT	1988
2	Tahitian Tall (TAT)	091	WAT	1969	091	WAT	1988
3	Rangiora Tall (RGT)	091	WAT	1969	091	WAT	1988
4	Cambodia Ream Tall (KAT07)	101	WAT	1970	091	WAT	1988
5	Cambodia Sre Cham Tall (KAT08)	101	WAT	1970	091	WAT	1988
6	Rotuma Tall (RTT)	101	WAT	1970	081	WAT	2002
7	Tonga Tall (TONT)	101	WAT	1970	081	WAT	2002
8	Tagnanan Tall (TAGT)	102	WAT	1975	081	WAT	2002
9	Vanuatu Tall (VTT)	111	WAT	1970	081	WAT	2002
10	Sri Lanka Tall (SLT)	112	WAT	1972	081	WAT	2002

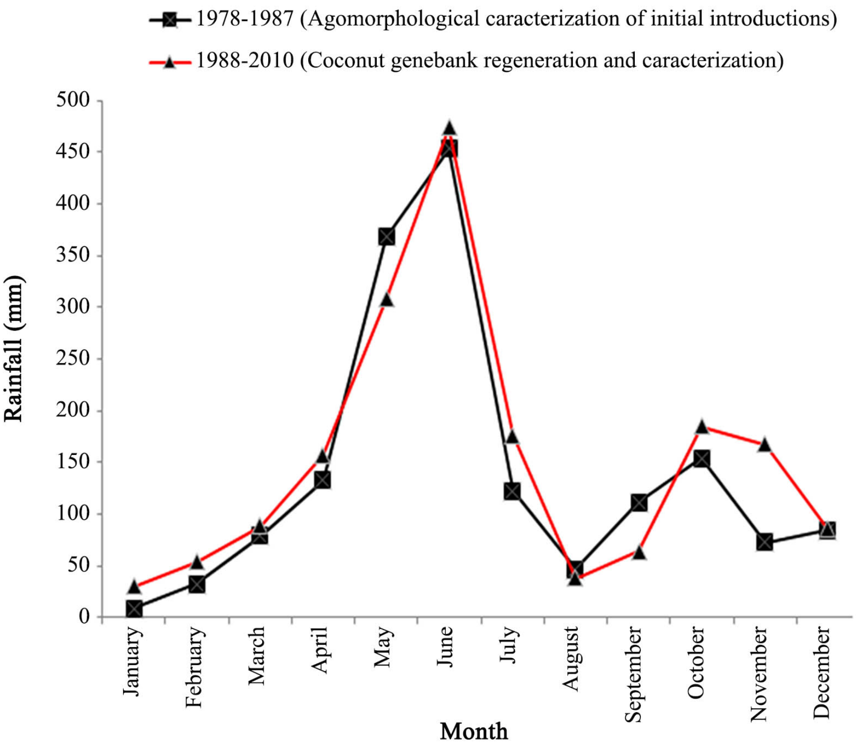

Figure 1. Means of monthly rainfall evolution on Marc Delorme station between 1978 and 2010.

2.2. Sampling and Measure of Agromorphological Traits

For each character, 30 or 300 coconut palms were observed respectively per accession and generation. Data about parental accessions were provided from Coconut Data Management (CDM) software of the Marc Delorme station. Regenerated coconut palms were observed from July 2009 to July 2011. Agromorphological data was recorded according to methods described [12] [13]. Twenty-six quantitative characters appearing in the coconut's descriptor and selected in long list of coconut descriptor study in detail by Yao were assessed on the stem (3), leaf (6), inflorescence (5) and the fruit (12) [14] [15].

On the stem, the stem girth at 20 cm above soil level (C20), the stem girth at 150 cm from the ground (C150) and the number of leaf-scars between 1 and 2 m from the ground (NLS) were measured.

Leaf measures concerned the leaves of rank 24. So the rachis length (RL), petiole length (PL), number of leaflets down one side of the leaf (NL), leaflet length (LL), leaflet width (LW) were measured on the leaf. The theoretical leaf area was calculated according to the mathematical formula ($LA = 2 \times NL \times LL \times LW$) stated by [16].

Floral measures were carried out in inflorescences raised by rank 24 leaves. The length of inflorescence axis (ILA), peduncle length (ILP), spikelet length (ILS), distance between point of spikelet insertion and insertion of the first fruit (ISFFD) and the number of spikelets (INS) were assessed.

Concerning fruits traits, Fruit Components Analysis (FCA) and yield characters were assessed. On the Four mature nuts were collected per bunch from one palm and stored for 15 days to achieve maturation stages before analysis of their components at laboratory. The weights of the whole fruit (FW), husk (HW), husked nut (HNW), shell (SW), endosperm (EW), water (WW) and the ratio of copra per nut (Q) were analyzed. Yield characters were also determined including the copra weight per nut (CNW = dry endosperm weight × 100/94). Fresh meat oil content (FMO) and dry meat oil content (DMO) were assessed based on weight of total oil extracted using Soxhlet method. While, the number of bunches per palm per year (NBPY) and the number of fruits harvested per palm per year (NFPY) were determined at the field.

2.3. Statistical Analyses

Weighted mean data of all characters collected from the Tall coconut accessions were used in all analyses. Means of raw data of each character were transformed from geometric weighting method [17] [18]. This transformation based on the performance of the experimental control West African Tall (WAT) was performed per generation. It permitted to minimize morphological gaps due to environment effects appeared between accessions planted on distinct plots during one generation. Indeed, WAT is considered as control in all experimental plots of Tall coconut palms at the Marc Delorme station. For each character, weighted means were done following relation [18]:

$$\bar{X}_{i,k} = \left(\bar{X}_{C,k} \times \bar{X}_{ij,k}\right) \Big/ \bar{X}_{Cij,k} \quad (1)$$

where $\bar{X}_{i,k}$ is weighted mean of the character *i* in generation *k* for one accession, $\bar{X}_{C,k}$ mean of the character *i* of the WAT control of all plots in generation *k*, $\bar{X}_{ij,k}$ raw value of the character *i* in plot *j* and generation *k* for one accession and $\bar{X}_{Cij,k}$ mean of the WAT control for the character *i* in plot *j* and generation *k*.

Principal Component Analysis (PCA) and Multiple Analysis of Variance (MANOVA) were performed using Statistica software v.7.1 [19]. PCA was conducted to identify divergence factors between G0 and G1 accessions generation. Likewise, MANOVA was achieved to reveal characters that discriminate generations G0 and G1 at 5% likelihood. Prior, the equality of variances of studied characters in accessions per generation was verified.

3. Results

3.1. Divergence Factors between Parental and Regenerated Accessions

Principal Component Analysis (PCA) achieved from the 26 agromorphological traits identified 3 main factors of divergence between parental and regenerated accessions (**Table 2**). The first three factors of PCA expressed together 69.95% of total variation.

Factor 1 explained 35.22% of the total variability (**Table 2**). Considering correlation values between axes and traits exceeding 0.7, Factor 1 was negatively

Table 2. Eigen values, total variance rate and correlation matrix between traits analyzed and the three principal components of the PCA.

	Divergence factors		
	First	Second	Third
Eigen value	9.15	5.24	3.78
Total variance (%)	35.22	20.17	14.56
% Cumulative	35.22	55.39	69.95
Stem traits			
C20	−0.37	**0.73**	0.30
C150	−0.41	**0.81**	−0.31
NFS	0.16	**−0.83**	0.33
Leaf traits			
PL	**−0.82**	0.19	0.33
NL	−0.26	0.63	0.12
RL	−0.62	0.37	−0.57
LL	−0.42	0.35	−0.70
LW	−0.27	**0.79**	0.34
LA	−0.61	0.69	−0.22
Inflorescence traits			
ILP	−0.63	−0.19	0.52
ILA	−0.64	−0.40	−0.19
ILS	−0.41	−0.63	−0.10
ISFFD	−0.50	−0.40	0.06
INS	−0.10	0.27	0.05
Fruit traits			
FW	**−0.93**	−0.26	−0.02
HNW	**−0.82**	0.09	0.15
SW	**−0.93**	−0.20	−0.04
HW	**−0.76**	−0.03	0.30
WW	**−0.74**	−0.42	−0.21
EW	**−0.89**	−0.35	−0.14
Q	0.27	−0.37	−0.52
CNW	**−0.80**	−0.38	−0.18
FMO	**0.72**	0.09	0.38
DMO	0.02	−0.12	0.51
NBPY	−0.51	−0.02	**0.73**
NFPY	−0.22	0.13	**0.79**

Variables mostly correlated to the components are shown in bold.

correlated with petiole length (PL; r = −0.82) and weights of whole fruit (FW; r = −0.93), husk (HW; r = −0.76), husked nut (HNW; r = −0.82), shell (SW; r = −0.93), water (WW; r = −0.74), endosperm (EW; r = −0.89) and coprah per nut (CNW; r = −0.8). Oil content in fresh meat (FMO) was positively correlated (r = 0.72) with Factor 1. Axe 1 was interpreted as divergence factor following components of the nut.

Factor 2 described 20.17% of the variance (Table 2). It was positively correlated with stem girth at 20 cm above soil level (C20; r = 0.73) and stem girth at 150 cm height (C150; r = 0.8) and negatively with number of leaf-scars (NLS; r = −0.81). It was defined as the divergence factor describing height growth and stem vigour.

Factor 3 explained 14.56% of the total variance (Table 2). It was positively correlated with number of bunches per palm per year (NBPY; r = 0.73) and number of fruits harvested per palm per year (NFPY; r = 0.79). Axe 3 was considered as divergence factor according to bunch and fruit yields.

3.2. Description of the Accessions Generation following Divergence Factors

The analysis of the parental and regenerated accession couples distribution has been achieved on the two plans formed by the Factors 1 - 2 and 1 - 3. The divergence Factors 1 and 2 explained together maximum of variability of 55.39% (Figure 2). They permitted to distinguish the two accessions generations. In the plan 1-2, majority of the parental accessions G0 were located in the negative part of the axis 1 and positive part of the axis 2.

Regenerated accessions G1 were located in the positive part of the axis 1 and negative part of the axis 2. So, the regenerated accessions diverge to the parental ones according to a low weight of nut components. Likewise, the regenerated accessions G1 are characterized by low stem vigour and less quickly growth.

The divergence Factors 1 and 3 explained together 49.78% of the total variability (Figure 3). Differentiation between the two Tall coconut generations G0 and G1 was less clear in plan 1-3.

3.3. Discriminate Traits of the Parental and Regenerated Accessions

Multiple analysis of variance (MANOVA) showed that only 8 (31%) of the 26 characters assessed, discriminate (F = 254.3; p = 0.049) G0 and G1 generations. Thus, characters that allowed a complete distinction of the two generations of Tall coconut palms are those describing bulb and stem girths, number of leaf-scars, petiole length, leaflet width, theoretical leaf area, the annual yield of bunches and nuts per palm (Table 3). That represents 100%, 50% and 17% of characters measured respectively on the stem, leaf and nut. In the majority, characters estimated essentially on the inflorescence and the fruit components didn't vary significantly after one regeneration cycle.

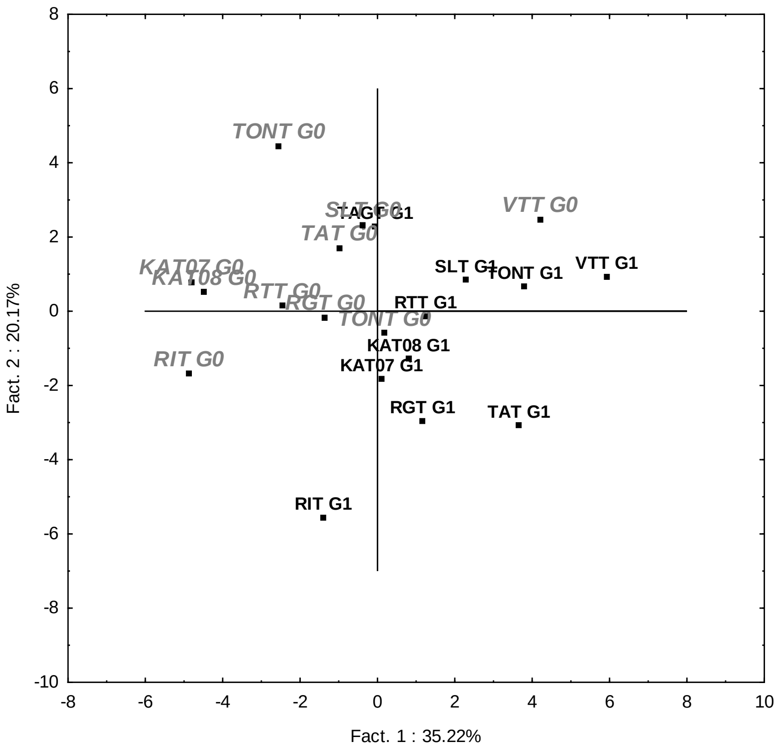

Figure 2. Parental (G1) and regenerated (G1) Tall coconut accessions on the first and second factor components. Factor loadings of components are listed in Table 3.

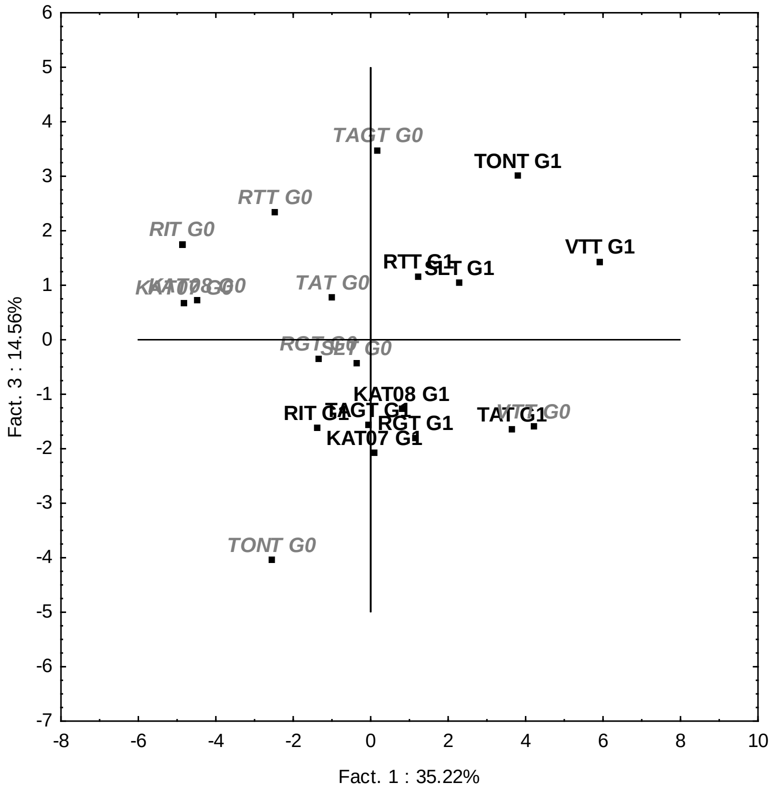

Figure 3. Parental (G1) and regenerated (G1) Tall coconut accessions on the first and third factor components. Factor loadings of components are listed in Table 3.

Table 3. Variation in the traits of each Tall coconut generations.

	Weighted means			
Characters (unit)	Parental generation G0 (N = 10)	Regenerated generation G1 (N = 10)	t	p
Stem traits				
C20 (cm)	196.67 ± 11.28	153.39 ± 21.88	30.91	<0.001
C150 (cm)	97.55 ± 7.29	88.25 ± 4.91	11.18	0.003
NLS	12.97± 2.02	14.90 ± 1.65	5.45	0.031
Leaf traits				
PL (cm)	157.83 ± 11.92	142.09 ± 4.42	15.29	0.001
NL	118.49 ± 3.54	117.92 ± 4.29	0.11	0.745
RL (cm)	436.87 ± 34.01	409.75 ± 23	4.36	0.051
LL (cm)	126.78 ± 8.63	121.38 ± 5.89	2.66	0.119
LW (cm)	6.37 ± 0.20	5.82 ± 0.68	5.93	0.025
LA (m^2)	19.21 ± 2.24	15.39 ± 1.46	20.37	<0.001
Inflorescence traits				
ILP (cm)	62.84 ± 5.90	58.61 ± 3.46	3.82	0.066
ILA (cm)	43.65 ± 3.42	42.36 ± 3.54	0.69	0.416
ILS (cm)	49.52 ± 5.16	49.65 ± 5.26	0.003	0.954
ISFFD (cm)	8.92 ± 1.14	7.99 ± 1.24	2.99	0.100
INS	43.70 ± 5.52	40.93 ± 4.39	1.54	0.230
Fruit traits				
FW (g)	1434.8 ± 283.65	1250.1 ± 252.21	2.36	0.141
HNW (g)	982.57 ± 214	845.15 ± 209	2.10	0.163
HW (g)	449.66 ± 87.25	399.26 ± 47.46	2.57	0.125
SW (g)	220.79 ± 37.16	186.99 ± 37.22	4.12	0.057
EW (g)	483.44 ± 79.33	413.93 ± 94.61	3.16	0.091
WW (g)	281.33 ± 110.31	268.30 ± 121.20	0.063	0.804
Q (%)	23.93 ± 1.63	25.08 ± 2.11	1.84	0.190
CNW (g)	273.14 ± 35.59	248.61 ± 51.87	1.52	0.233
OFM (%)	36.01 ± 1.70	37.92 ± 3.18	2.80	0.111
ODF (%)	65.73 ± 3.51	66.88 ± 3.85	0.493	0.491
NBPY	9.46 ± 2.82	6.29 ± 0.93	11.34	0.003
NFPY	48.01 ± 1.88	30.14 ± 8.42	7.47	0.013

N: number of accession per generation; p: probability value of t-test; t: t-test statistical value; G0: parental population of coconut palm; G1: first cycle of regenerated population of the coconut palm.

4. Discussion

Agromorphological diversity analysis showed divergence factors between the two Tall accession generations. Regenerated accessions have low vegetative and production traits values compared with parental accession data. The recorded results showed that low number of studied characters allowed discrimination between G0 and G1 generations. These results demonstrated that after one regeneration cycle, coconut accessions were not well adapted ecologically. However, the evolutionary of the accessions during their regeneration would support the decrease of the expression of the morphological characters. Besides, the variations affected both all regenerated accessions and some characters studied. That suggests a common origin for similar modification of morphological characters after one regeneration cycle. Two hypotheses based on adaptability of the coconut palms could explain the decrease of the agromorphological characters values in regenerated accessions.

The first hypothesis would be low mineral nutrition level of the regenerated coconut palms. Indeed, in the experiments for characterization of the initially coconut accessions, palms were treated with good agronomic care and manure application [20]. However, at the experimental site, soil fertilization and plant sanitary maintenance were not regular in regenerated accessions. This situation could have reduced the yield potential of the material tested according to [21]. A similar reasoning has been considered by [22] to explain the lower collar circumference recorded on young coconut plants of PB121 variety in comparison with those obtained from previous experiments. Likewise, all plots of the regenerated coconut accessions were established on old plot planted with parental accessions and improved hybrids. Therefore, previous cultural involved progressive impoverishment of soil minerals. Moreover, we also noticed no more herbaceous leguminous such as *Pueraria* sp. and *Centrosema* sp. which were planted with parental accession in previous plots [20]. According to [23] and [24], fertilization of coconut palms planted at the field must be renewed very often to preserve a good mineral nutrition. It proved that unavailability of the soil mineral elements such as nitrogen, phosphor, potassium and magnesium disrupts coconut palms metabolism and agromorphological trait expressions [22]. For instance, the lack of potassium and mineralized nitrogen reduce coconut leaves emission as far as their lengths [25]. [26] indicated that lack of potassic manure causes a decrease of bunches and nuts yield in coconut palms. Lack of the assimilated phosphor disrupts the palms growth and reduces the length of the leaves [23].

The second hypothesis that explains the decrease of the vegetative and production characters' expression would be the adaptability of regenerated coconut palms in local environment. The monthly distribution of the rainfall remained similar since 1978 at the Marc Delorme research station (**Figure 1**). Thus, regenerated accessions developed less bulky bulbs than their parents and less dense roots for absorption of the water which is an abundant resource in our ecological conditions [27]. So, for the regenerated coconut trees, the expression of the stem vigour characters described by [16] as a drought tolerance trait has appeared useless. That could explain the regression of bulb girth which represents the water storage organ. The origin of leaf length reduction would be due to plant physiological states. Indeed, reduction of the foliar area would signify an adjustment of the leaf surface to minimize chlorophyllous reaction synthesis according to the reserves of water in the palm and soil minerals. The implication of the foliar characters in coconut palms' adaptability to the ecological conditions has been mentioned by [28]. Also, positive correlations between vegetative and production characters would explain the relative decrease of the fruit components characters in regenerated accessions as also mentioned by [20] and [21] in previous studies about Tall coconuts. The first authors explained the correlations between characters from different coconut palm organs by pleiotropy phenomenon. This situation appeared when one gene or group of genes induces simultaneously several characters expressions. These genes can induce a sequence of actions either through multiple interactions, or by acting physiological biosynthesis chains in the entire plant [20]. Moreover, [21] reported that the production of hormones by one organ, could initiate the development of another.

5. Conclusion

We could support those origins of the divergences between parental and regenerated accessions are not genetics but depend on ecological conditions where experimental plots were established. Despite this environmental influence, agro-morphological divergence between coconut generations is low. Considering the conservation of the expression of the majority of phenotypical traits in one regeneration cycle, regenerated accessions are able to be used for research and development purposes in Côte d'Ivoire as done with their parents.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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