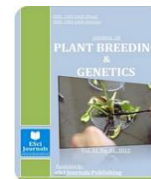




Available Online at ESci Journals

Journal of Plant Breeding and Genetics

 ISSN: 2305-297X (Online), 2308-121X (Print)
<http://www.escijournals.net/JPBG>


TYPOLOGY IN THE FERTILITY OF SEVEN TALL COCONUT (*COCOS NUCIFERA* L.) POPULATIONS USING ARTIFICIAL POLLINATION TRAITS IN FIELD GENE BANK

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ABSTRACT

This study investigates the fertility typology for seven Tall coconut accessions using artificial pollination characters. Ten traits describing fertility were considered for characterizing three groups of coconut individuals per accession. Ten males parental, twenty to forty female parental, and ten progenies were randomly explored per coconut accession. Except for the rate of seeds germination, the outcomes revealed the usefulness of the characters assessed for the discrimination of the accessions studied. The typology of the fertility of the accessions studied is independent of the origins and highlighted two main groups. The first group gathers Laccadive Common Tall (LCT) and West African Tall (WAT and WAT6) accessions including the most fertile male parents and the females showing great production of seed nuts. From this group, the progenies set out a lack of vigour. The second group is constituted of Panama Tall (PNT01 and PNT02), Solomon Island Tall (SIT) and Gazelle Peninsula Tall (GPT) accessions with characters opposite to the previous ones. The investigations of the Tall coconut accessions fertility are fundamental to address the low size of genitors and progenies for respective field genebank regeneration and representative field replanting.

Keywords: Tall coconut, controlled pollination, fertility, field genebank regeneration.

INTRODUCTION

With 12 million hectares, *Cocos nucifera* L. (*Areaceae* family) is the most cultivated plant over the world, yielding in 5.8 million tons of copra.year⁻¹ (Soizick, 1984; FAO and Oil Word, 2010). It's a perennial oleaginous crop growing in wet tropical lands. This plant record two different types of cultivar, namely allogamous "Tall" trees and autogamous "Dwarf" trees (Sangaré *et al.*, 1978). From Western Africa, 145.5 million tons of copra are produced each year, from 128, 500 hectares (ha) of coconut fields, and remain livelihoods for 100, 000 families (Ouvrier *et al.*, 1995). In Côte d'Ivoire, the coconut is cultivated on 50, 000 ha with a yield of 55, 000

tons of copra.year⁻¹ (Konan *et al.*, 2006). On the coastal region of this country, 20, 000 families make their main income from coconut products (Assa *et al.*, 2006).

The attempts on coconut gene improvement at the Marc Delorme Research Station in Côte d'Ivoire consist of investigations about the variability of the plant material gathered from the whole inter-tropical region and kept alive at a field. The *ex-situ* gene bank, rich of 99 coconut accessions, is set up as in International Coconut Genebank for Africa and the Indian Ocean (ICG-AIO) since 1999 (Konan, 2005). Such a method of dynamic management of the germplasm faces major concerns. Indeed, the fast growth of many coconut populations, specifically Tall ecotypes beyond 20 years after planting, raises the difficulties to overcome during their exploitation for the research. At that age, the coconut leaves crown are so higher and hardly accessible that breeding processes

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can't be achieved in safety conditions. Consequently, the working of the breeding program seems to be compromised. The mitigation plan drawn by the germplasm keeping managers is the renewal of the old accessions previously introduced. Regeneration is based on the choice of a sounded number of male and female individuals, within the parental accessions, to be crossed by controlled pollination process (De Nuce *et al.*, 1980). The practice requires the contribution of the pollen produced from interesting male coconut parental on the mature female flowers of seed-bearing trees in isolated condition using pollination jute-bags. Otherwise, the coconut records a low rate of breeding since 20-30 nuts.tree⁻¹ and 0-4 nuts.tree⁻¹ are yearly produced in respective natural and controlled pollinations (De Nuce *et al.*, 1980; Yao *et al.*, 2010). This low rate of reproduction delays the regeneration achievements and increases the financial costs involved. It also affects the representativeness of the field regenerated accessions and constitutes one of the major gene drift factors during the regeneration process of the plant germplasm (Johnson *et al.*, 2004). Previous attempts on the controlled pollination have been achieved regarding the setting of pollination method (De Nuce *et al.*, 1980), and the extraction and preservation of the pollen (Rognon and De Nuce, 1978). Hence, Yoboue (2009) and Yao *et al.* (2010) respectively revealed that the isolating inflorescence bag and the pollen's quality result in significant effects upon the success of the controlled

pollinations. Yet, there's scanty survey delivered specifically for the regenerative performance in controlled pollination of a high number of coconut ecotypes. The current study aims to search relevant traits for measuring the regenerative performance of the coconut in controlled pollination and also targets the morphological divergence between the plants accessions according to the traits deriving from the breeding performance. The outcome of the fertility typology of these accessions could help in designing efficient strategy regarding the effective size of parental individuals to be considered per accession to improve the regeneration program.

MATERIAL AND METHODS

Plant material: The survey was achieved on seven (7) Tall coconut accessions of the field gene bank in regeneration process at the Marc Delorme Research Station for coconut (5°14.5' North latitude and 3°54.5' West longitude) of the National Centre of Agriculture Research (CNRA) in Côte d'Ivoire (Table 1). The coconut accessions were constituted of West African Tall (WAT, WAT6), Panama Tall (PNT01, PNT02), Gazelle Peninsula Tall (GPT), Solomon Island Tall (SIT), and Laccadive Ordinary Tall (LCT). The studies were carried out during two consecutive years, 2005 and 2006. From both years, mean temperature and rainfall were respectively of 26.4°C and 1643 mm. The means of sunshine and the relative hygrometry were respectively of 2304 hours and 85.8%.

Table 1. Some characteristics of the seven Tall regenerated accessions of *Cocos nucifera* L. studied.

Accession	Population	Code	Origin	Location
West African Tall	Côte d'Ivoire	WAT	West Africa	Marc Delorme Station
West African Tall	Ouidah	WAT6	West Africa	Marc Delorme Station
Panama Tall	Aguadulce	PNT01	Latin America	Marc Delorme Station
Panama Tall	Monagre	PNT02	Latin America	Marc Delorme Station
Gazelle Peninsula Tall	Gazelle	GPT	South Pacific	Marc Delorme Station
Solomon Island Tall	Saraoutou	SIT	South Pacific	Marc Delorme Station
Laccadive Ordinary Tall	Laccadive Ordinary	LCT	Indian Ocean	Marc Delorme Station

METHODOLOGY

Standard descriptors for controlled pollinations: The measures cast in the study regarded 10 descriptors of which eight (8) were selected among the coconut usual descriptors listed by IPGRI (1995). They consisted of the number of days between opening of two consecutive inflorescences (DSI), number of spikelets per inflorescence (INS), number of female flowers per

inflorescence (INFF), rate of fruits bearing (RFB), rate of seeds germination (RSG), number of leaves produced per seedling (NL), height of seedlings (SH) and collar girth of seedlings (CGS). The remaining 2 descriptors were cast out of the formal list, namely the weight of pollen per inflorescence (IWP) and the rate of pollen *in vitro* germination (RPG). From overall coconut accessions, assessment of artificial pollination traits was achieved

upon 3 groups of individuals, specifically the male parental individuals, the female parental individuals, and their progenies bred in the nursery.

Assessment of the fertility in coconut male parental plants:

The number of spikelets/inflorescence, the weight of pollen/inflorescence, and the pollen viability shown by the rate of the pollen *in vitro* germination were the descriptors assessed for the fertility. Per coconut accession, ten male parental plants were randomly selected and the number of spikelets (INS) determined at field upon the rank 10 new-opened inflorescences borne by the trees. Then, the pollen was extracted using the process drawn by Rognon and De Nucé (1978) and the full pollen gathered from each inflorescence was weighed (IWP) using a two-digit scale. For the determination of the rate of pollen *in vitro* germination (RPG), 0.25 g of pollen was plated in solid media plates prepared with agar, sucrose and distilled water (Yao *et al.*, 2010). The number of germinated or not germinated grains of pollen was observed under Light Microscope (LM) equipped of a camera VC 3011 at 100x magnification (Yao *et al.*, 2017). A grain of pollen has germinated when its diameter was inferior or equal to the length of the pollen tract (Yao *et al.*, 2010).

Assessment of the fertility in coconut female parental plants:

At the field, two (2) to four (4) female parental coconuts were randomly selected per accession and fertilized with the pollen collected from the male parental chosen within the same Tall coconut population according to the method suggested by De Nuce *et al.* (1980). At all, 20 to 40 female parental individuals were processed per accession. The mean delay between two consecutive opening inflorescences was determined along with the development of 2 to 4 consecutive inflorescences from each female parental individual, and the number of days recorded. Then, the female flowers borne per inflorescence (INFF) and the mature seeds on per bunch (NMS) were respectively counted at the earlier controlled pollination implementation and 12 months afterwards. Finally, the rate of successful fruit-bearing (RFB) resulting from the controlled pollination was calculated:

$$\text{RFB (\%)} = \frac{\text{NMS} \times 100}{\text{INFF}}$$

Assessment of vegetative vigour from coconut progenies in nursery: Ten mature seeds, harvested from

female fruit bearing coconuts deriving from the controlled pollination process within each accession, were randomly selected and observed. These seeds were kept in a seedbed and processed for germination according to the method described by Wuidart (1981a and b). Thereafter, the rate of seeds germination (RSG) was calculated using the number of seeds germinated (NSG) and the total number of seeds sowed (NSS):

$$\text{RSG (\%)} = \frac{\text{NSG} \times 100}{\text{NSS}}$$

At 3 months age of seedlings, the number of leaves (NLS), the height (SH), and the collar girth (CGS) were determined in the nursery from the seedlings.

Statistical analysis: The data were statistically treated using Statistica software v.7.1 (StatSoft Inc., France). The raw data were previously sounded for statistical normality using the Shapiro λ -Wilk test (λ -Wilk <0.05). The descriptors displaying λ -Wilk value over 0.05, (rate of fruit-bearing and weight of pollen/inflorescence) were processed with decimal logarithmic functions ($\text{Log}_{10}(x+1)$ and $\text{Log}_{10}(x)$, respectively) for their normalization. Then, a one-way analysis of variance (ANOVA-1) including Student Newman Keuls (SNK) post-ANOVA comparison test were performed regarding the accessions for identification of the relevant fertility descriptors. Genetic similarities between coconut accessions regarding the full controlled pollination fertility were assessed by drawing multivariate design, namely Principal Components Analysis (PCA) and Hierarchical Cluster Analysis (HCA) using averages recorded from the overall descriptors measured. The bond and aggregation options of the accessions clusters consisted of the Euclidian distances and the UPGMA method, respectively (Dagnelie, 2003).

RESULTS

The fertility of the groups of male and female parental individuals and their progenies:

The ANOVA-1 statistical test evidenced a significant effect of the accession on the whole characters assessed, except for the rate of seeds germination in the nursery (Table 2). The SNK tests revealed various homogeneous accessions groups according to the artificial pollination descriptors assessed from the male and female parental plants and their progenies (Table 3).

For the male individuals, the WAT6 and SIT accessions provided respectively highest (33 ± 14) and lowest (22 ± 5)

mean numbers of spikelets per inflorescence. The greatest values from the weight of pollen per inflorescence were recorded with the LCT (22.15±15.11 g), the WAT6 (12.90±5.76 g), and the GPT (10.73±4.1 g). The PNT01 produced a lower amount of pollen with a mass of 5.41±1.29 g, whereas the WAT, PNT02, and SIT

accessions recorded intermediate amounts fluctuating between 8.06±3.02 and 9.58±2.96 g. The rate of the *in vitro* germinating pollen was higher for WAT6 (37.2±3.4%) and LCT (36.39±3.35%) accessions compared the values of 30.36±3.4% and 30.83±3.71% respectively provided from PNT02 and PNT01 (Table 3).

Table 2. Analysis of variance of 10 artificial pollination traits in 7 Tall accessions of *Cocos nucifera* L. at Côte d'Ivoire coconut gene bank.

Artificial pollination traits	df	Mean of squares	F-value	P-value
INS	6	135.34	2.88	0.015
IWP (g)	6	0.14	3.26	0.007
RPG (%)	6	72.62	3.74	0.003
DSI (days)	6	85.06	4.82	p<0.001
INFF	6	174.61	4.68	0.001
RFB (%)	6	0.46	6.05	p<0.001
RSG (%)	6	825.82	1.55	0.177
NL	6	7.16	6.93	p<0.001
CGS (mm)	6	13.69	4.05	0.002
SH (cm)	6	5196.11	10.75	p<0.001

INS: Number of spikelet per inflorescence; IWP: Weight of pollen per inflorescence; RPG: Rate of pollen *in vitro* germination; DSI: Number of days between openings of two consecutive inflorescences; INFF: Number of female flower per inflorescence; RFB: Rate of fruit-bearing; RSG: Rate of seed germination; NL: Number of leaves produced; CGS: Collar girth of the seedlings; SH: Seedling height; df: degree of freedom.

Table 3. Comparison of means of the fertility of 7 Tall accessions of *Cocos nucifera* L. from 10 artificial pollination traits in Côte d'Ivoire coconut gene bank.

Group of individuals	Artificial pollination traits	Value per accessions (mean ± standard deviation)						
		WAT	WAT6	PNT01	PNT02	GPT	SIT	LCT
Male parents	INS	25.00±4.00ab	33.00±14.00a	26.00±5.00b	30.00±5.00ab	24.00±1.00ab	22.00±5.00b	29.00±7.00ab
	IWP (g)	9.58±2.96ab	12.9±5.76a	5.41±1.29b	8.41±2.88ab	10.73±4.10a	8.06±3.02a	22.15±15.10a
	RPG (%)	33.39±5.64ab	37.2±3.34a	30.83±3.71b	30.36±6.33b	35.65±2.58ab	32.76±4.57ab	36.39±3.35a
Female parents	DSI (days)	34.00±5.00a	25.00±2.00b	30.00±3.00ab	27.00±3.00b	27.00±3.00b	30.00±4.00ab	31.00±5.00ab
	INFF	21.00±4.00a	18.00±4.00a	10.00±3.00b	9.00±3.00b	16.00±4.00ab	16.00±12.00ab	15.00±4.00ab
	RFB (%)	5.78±2.80b	9.32±9.81b	5.52±5.22b	16.84±11.22a	7.30±4.90b	4.87±2.61b	17.40±5.78a
Progenies	RSG (%)	65.56±22.34	56.28 ±27.9	57.43±18.14	72.11±25.81	69.56±22.71	66.79±28.8	82.82±10.37
	NL	6.00±1.00b	5.00±1.00b	9.00±1.00a	9.00±1.00a	9.00±1.00a	9.00±1.00a	5.00±1.00b
	SH (cm)	78.90±24.14bc	65.10±14.90cd	98.00±20.13ab	103.80±36.60ab	93.40±17.37ab	117.40±21.22a	52.30±8.71d
	CGS (mm)	9.60±2.12ab	9.60±1.50ab	10.40±2.06ab	9.80±1.98ab	11.70±0.82a	11.10±2.64a	8.10±1.00b

Per descriptor, values indexed with different letters are significantly different. INS: Number of spikelet per inflorescence; IWP: Weight of pollen per inflorescence; RPG: Rate of pollen *in vitro* germination; DSI: Number of days between openings of two consecutive inflorescences; INFF: Number of female flower per inflorescence; RFB: Rate of fruit-bearing; RSG: Rate of seed germination; NL: Number of leaves produced; CGS: Collar girth of the seedlings; SH: Seedling height.

Results about female parental trees reproductive success showed that the inflorescences developing

frequency was faster from the WAT (34±5) and slower with WAT6 (25±2), PNT02 (27±3) and GPT (27±3). The

WAT6 and WAT accessions developed inflorescences bearing a higher number of female flowers (18 ± 5 and 21 ± 5 , respectively) compared to the lower values from PNT02 (10 ± 3) and PNT01 (9 ± 3), and even the observations from GPT, LCT, and SIT (15 ± 4 to 16 ± 12). The rate of fruit-bearing differentiated the greater values resulting from LCT and PNT02 accessions ($17.4\pm 5.78\%$ and $16.84\pm 11.22\%$, respectively) and the low rates of fruit borne by the WAT6, GPT, WAT, PNT01, and SIT accessions ($4.87\pm 2.61\%$ to $9.32\pm 9.81\%$) as shown in table 3.

Regarding the progenies in the nursery, the rates of seeds germination varied between 56.28 ± 27.9 and $82.82\pm 10.37\%$ within the Tall coconut accessions studied. Up to 3 months of age, the mean number of leaves set out in nursery showed was superior from GPT, PNT02, PNT01 and SIT accessions (9 ± 1 leaves) compared to the WAT, WAT6, and LCT (5 ± 1 to 6 ± 1 leaves). The seeds produced by SIT accession resulted in the highest seedlings measuring 117.4 ± 21.22 cm against 52.3 ± 8.71 cm for the lower height seedlings got from LCT accession. SIT and GPT developed seedlings with higher collar girth (11.7 ± 0.82 cm and 11.1 ± 2.64 cm) than those of LCT (8.1 ± 0.99 cm). Intermediate values of collar girth varying between 9.6 ± 1.6 and 10.4 ± 2.06 cm were recorded from the PNT01, PNT02, WAT, and WAT6 (Table 3).

Genetic similarities between Tall coconut accessions from the descriptors assessed: From the Principal Components Analysis (PCA), the 2 top components (F1 and F2) displayed respective Eigenvalues of 4.67 and 2.33 and both cumulated contribution of 70.09% (Table 4). The first component (F1) solely explained 46.77% of the total variability and was positively correlated with the weight of pollen per inflorescence (IWP) and negatively with to the number of leaves produced (NL), the collar girth (CGS) and the height of seedlings (SH) of seedlings in the nursery. The second component (F2) supported 23.31% of the total variability and insured significant positive correlation with the rate of fruit-bearing (RFB) and negative correlation with the number of female flowers per inflorescence (INFF). Figures 1 and 2 illustrate the 7 Tall coconut accessions clustering from respective F1-F2 PCA design and CHA dendrogram afforded for structuring the variability regarding the fertility descriptors of the coconuts accessions studied. Hence, the first group was constituted of LCT, WAT6, and WAT accessions, whereas the second group gathered the PNT01, PNT02, SIT and GPT coconut accessions.

Table 4. Factor loadings of each artificial pollination traits in the first three-factor components.

Components or Factors	F1	F2
Eigenvalues	4.67	2.33
Variance (%)	46.77	23.31
Cumulative variance (%)	46.77	70.08
INS	0.60	-0.49
IWP	0.92	-0.004
RPG	0.66	0.37
DSI	0.04	0.60
INFF	0.31	0.87
RFB	0.65	-0.71
RSG	0.47	-0.30
NL	-0.79	-0.40
CG	-0.86	0.13
SH	-0.93	-0.15

INS: Number of spikelet per inflorescence; IWP: Weight of pollen per inflorescence; RPG: Rate of pollen in vitro germination; DSI: Number of days between openings of two consecutive inflorescences; INFF: Number of female flower per inflorescence; RFB: Rate of fruit-bearing; RSG: Rate of seed germination; NL: Number of leaves produced; CGS: Collar girth of the seedlings; SH: Seedling height.

DISCUSSION

Overall morphological markers assessed differentiated the accessions except for the rate of seeds germination. This result reveals the importance of the morphological descriptors in the characterization investigations as mentioned by Gopal *et al.* (2008) about the assessment of such types of markers to discriminate varieties of potato. The markers such as the rate of *in vitro* germinating pollen and the weight of pollen per inflorescence that do not appear in the coconut conventional descriptors listed by the IPGRI (1995) were very discriminative. Similar results were previously mentioned by Yao *et al.* (2010) from both natural "Tall" and "Dwarf" coconut ecotypes. However, the rate of seeds germination could be deleted while measuring the breeding performance in controlled pollination of any coconut accession. The Discriminant ability of this descriptor would be due to the mature stage of the seeds kept on seedbed and used for regenerating the coconut accessions. For overall coconuts studied, these seeds are harvested from bunches offering at least a brown epidermis walnut as significant proof of similar physiological maturity stage (Wuidart, 1981a, Harries, 2012).

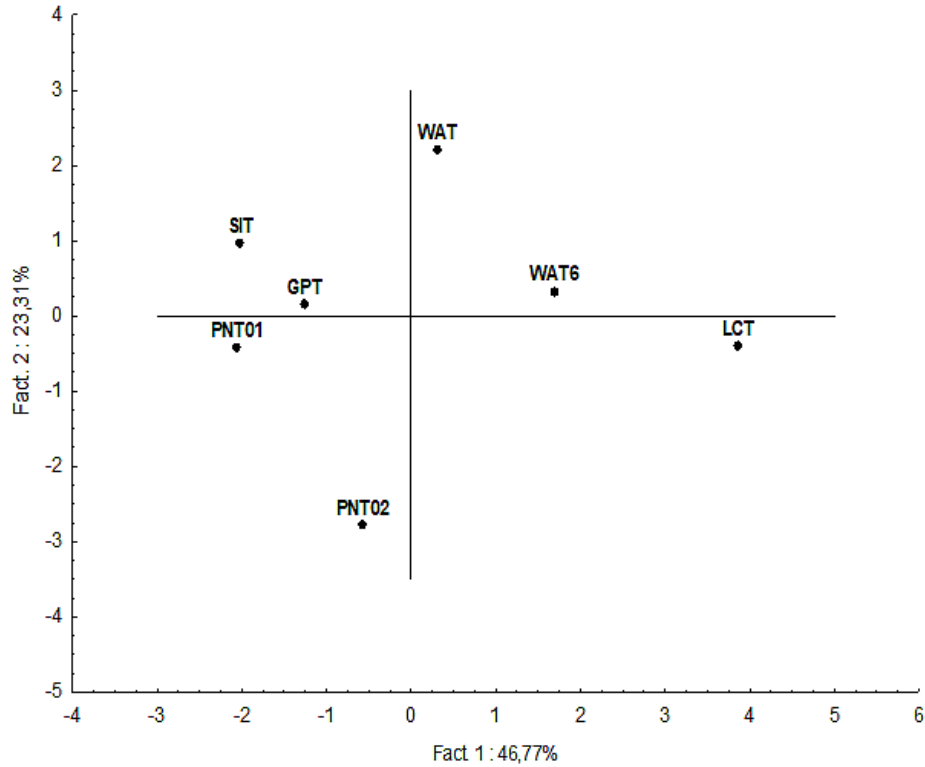


Figure 1. Repartition of the 7 Tall accessions of *Cocos nucifera* L within the plan 1-2 of PCA performed from artificial pollination traits.

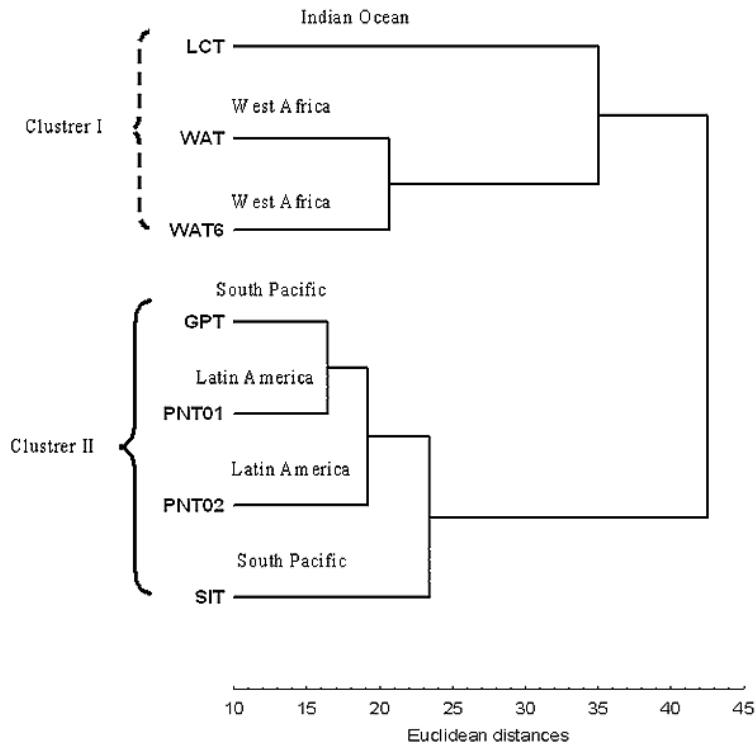


Figure 2. Dendrogram UPGMA illustrating the bonds between 7 Tall accessions of *Cocos nucifera* L. according to the artificial pollination traits.

The variability with the breeding performance of the coconut accessions would be in genetic nature since they are gathered from various geographical areas and planted in the same environmental and experimental conditions. At all, these results show that the 9 discriminative characters identified are very efficient to estimate the breeding performance as well as to study the gene variability. Such an arguing was assumed by Guetet *et al.* (2008) to deduce the morphological markers related to the breeding strength from natural populations of *Allium roseum* in Tunisia.

The means comparing tests emphasized a great divergence between the accessions studied according to the characters assessed from the male and female parental individuals and the progenies. Indeed, the Tall coconuts are very heterogeneous following their allogamous reproduction system (Sangare *et al.*, 1978). With the PCA analysis, the coconut accessions studied were distributed in two principal groups according to the agro-morphological characters relating to the artificial pollination traits. The first group was composed of the accessions presenting the best male parental individuals as well as the individuals showing good pollen production abilities, specifically the LCT, WAT6 and WAT accessions. These coconuts produce a great number of seeds, but the resulting seedlings were frail. They should, therefore, care in the nursery because the lack of vigour could impede the coconut transplanting at the field. The second group gathered the 4 remaining accessions PNT01, PNT02, SIT and GPT with the most vigorous progenies. From both groups, WAT and PNT02 have the particularity to provide respectively lower and higher rates of fruit-bearing. This result could suggest that the local ecotypes do no more lead to a fulfilled result in all controlled pollination. Any successful large production of seeds from these ecotypes needs to work a higher number of controlled pollination.

Also, various originated coconut accessions are gathered into the same group. They consist in the group of Afro-Indian populations (WAT, WAT6 and LCT) and the group of Latin America and South Pacific populations (PNT01, PNT02, SIT and GPT). The classification corroborates morphological clustering of Tall coconut accessions From International Coconut Genebank for Africa and Indian Ocean (ICG-AIO) as reported by Konan (2008) and Yao *et al.* (2019). Thus, the present study revealed that from two genetic pools into ICG-AO they are the coconut accessions accounting the best male parental individuals

and the ones with good seed production abilities under artificial regeneration. Other studies showed the effect of the physiological condition and the pollen's fertility upon the efficiency of seeds in the artificial pollinations (Charrier, 1990; Colas and Mercier, 2000; Yao *et al.*, 2010; Yao *et al.*, 2017).

CONCLUSION

This work aimed to probe the typology of the fertility in controlled pollination of seven accessions of Tall coconut in Côte d'Ivoire using ten descriptors of pollination. Overall descriptors assessed have discriminated the coconut accessions except for the rate of seeds germination in the nursery. The LCT, WAT6, and WAT accessions include the most fertile male parental plants and the best seeds production plants but result in frail progenies. Oppositely, the group of PNT01, PNT02, SIT, and GPT accessions presents low fertile male and female parental individuals but providing vigorous progenies. These behaviour differences between the accessions might be accounted in the programs of regeneration and preservation of the coconut gene resources. The knowledge gathered from the fertility of these Tall coconut accessions could, therefore, be considered to address the low size of progenies to be transplanted at the field for ensuring their representativeness during regeneration. Thus, the gene drifts risks would be mitigated.

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