

Full Length Research Paper

Controlled pollination and representativeness of the parental genotypes used during the regeneration of old Tall coconut accessions (*Cocos nucifera* L.) from the International Collection for Africa and the Indian Ocean

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After 20 years of conservation of accessions of Tall coconut palms in the International Coconut Collection for Africa and the Indian Ocean (ICC-AIO), they become unusable because of the height of the coconut palms which makes variety creation operations difficult. The regeneration of old accessions was therefore undertaken. However, the representativeness of the parental palms used as male or female genitors during the regeneration cycles is not known. So, the success of the regeneration method in terms of the representativeness of the parental genotypes used as genitors has never been evaluated. This work aimed to characterize the of controlled pollination and method and to evaluate the representativeness of the parental genotypes used during the regeneration of the Tall coconut accessions. The results shown that to regenerate a Tall coconut accession with n individuals, 2.11 n controlled pollinations must be carried out. In addition, the analysis highlighted differences in the representativeness of the parental genotypes sampled during the regeneration process of a Tall coconut palm accession by controlled pollination. These differences in the representativeness of the parental genotypes are caused by the mortality in the field of the regenerated plants planted, the excessive height of certain parental genotypes as well as their low seed yield by controlled pollination. The evolution of the diversity level of the offspring from the parental genotypes used at each regeneration cycle showed a reduction. Nowadays, in regeneration programmes for *ex situ* or field collections of coconut palms or other perennial plants, it is essential to take pedigree into account when choosing the genitors to be used in crosses, in order to maintain maximum diversity in the regenerated generations of accessions.

Key words: *Cocos nucifera* L., tall ecotypes, *ex situ* collection, rejuvenation, Côte d'Ivoire.

INTRODUCTION

The coconut palm (*Cocos nucifera* L.) is an oilseed of the Arecaceae family present throughout the humid

intertropical zone. All parts of the coconut palm trees are valued in an artisanal and/or industrial way, but it is

mainly cultivated for its fruits. The kernel of the fruit is of great economic and commercial interest and gives the coconut palm its quality as an oleaginous industrial plant (Bourdeix et al., 2005).

Within the framework of research activities, 37 accessions of the Tall ecotype and 16 of the Dwarf ecotype were introduced into Côte d'Ivoire from intertropical zone and kept in the field between 1952 and 1982 (Fremont and De Nuce, 1971). The large diversity thus constituted has contributed to the erection of the collection, since 1999, into International Coconut Collection for Africa and the Indian Ocean or ICC-AO (Batugal and Jayashree, 2005). The collection is also classified as World Heritage by the Food and Agriculture Organization of the United Nations (FAO).

The exploitation of the variability of the plant material introduced has enabled the creation of the best-cultivated improved hybrids in the world (Bourdeix et al., 1992; Bourdeix et al., 2005).

After 20 years of conservation of the Tall coconut ecotypes, they become unusable because of the high growth in height of the coconut palms which have a height of more than 12 m. This height of the coconut palms makes varietal creation operations difficult. The regeneration of old accessions was therefore undertaken to have plant material to facilitate field interventions without too many risks. However, the representativeness of the parental genotypes during the regeneration cycles is not known and the regeneration method has never been evaluated. Indeed, the regeneration of the coconut palm collection in Côte d'Ivoire is the first of its kind in all the countries hosting an *ex situ* coconut palm collection and grouped within the COGENT network. Similarly, if the production of seed by the controlled pollination method is controlled, the implications of this method in maintaining the diversity level of regenerated accessions have not yet been studied. The objective of this study is to characterize the method of controlled pollination and to study the representativeness of the parental genotypes used to regenerate old Tall coconut accessions.

MATERIAL AND METHODS

Plant material

The work focused on the populations of parental genotypes from 22 regenerated Tall coconut palm accessions in the International Coconut Collection for Africa and the Indian Ocean located at the Marc Delorme Research Station in Port-Bouët some of which have already been studied morphologically by Yao et al. (2019). The plant material was distributed over six coconut plant genetic resources conservation plots (Table 1).

The causes of the differences in representativeness in relation to

the fertility and the height of the selected parents were sought from the populations of parents from five accessions of Tall coconut palm. These are WAT, PYT1, SNT, NGT4 and MZT accessions regenerated between 2005 and 2010 that had undergone two cycles of regeneration were studied. This choice takes into account their importance in the coconut tree improvement scheme and the data available on the genealogy of the progenies resulting from these parents.

Technical materials and nomenclature of plant material used

For the management of data from controlled pollinations, Visual FoxPro 6.0 software was used. The data relating to the pedigree of the parental genotypes are digitized in two tables or Dbase type files. These are the tables named "fertilization.dbf" and "allpalm.dbf" which respectively group information relating to the pedigrees of seeds from controlled pollinations and plants planted in the field (Figure 1). Using the Structured Query Language (SQL) of the Visual Foxpro software, Software programs have been developed to meet the needs of data management on regenerated accessions. These Software programs were designed for the management and monitoring of the pedigrees of individual regenerated palms per accession. They were developed in collaboration with CIRAD to manage the data generated within the artificial fertilization laboratory of the *Centre National de Recherche Agronomique* (CNRA).

A codification is used to identify the crosses or controlled pollination carried out and the genotypes (parents and progenies) in plantation. The controlled pollination number is characterized by 1 to 2 letters of the alphabet followed by 5 to 4 Arabic numerals. This is for example the number AN3482. The number of the male or female parent is composed of the letter P followed by 5 Arabic numerals. This is for example the parent P13373. The genotypes in the field are identified by a registration number which indicates their geographical coordinates in a given plot of the collection. The lines are numbered from West to East and the genotypes from South to North. Thus, the genotype number 062 01 02 occupies the second row on the first line to the south of plot 062 (Figure 1).

Elaboration of the list of parental genotypes

Two lists of parental genotypes used for regeneration have been developed. This is the list of parental genotypes used for the first regeneration cycle and that of the second regeneration cycle. The parental genotypes of the first cycle (g0) of regeneration constitute the parents of those of the second cycle (g1).

Therefore, a parental genotype from a second-generation accession has g1 parental genotypes as parents and g0 parental genotypes as grandparents. The lists were developed using the software program designed on Visual FoxPro software called genealo1.dbf. Figure 2 shows the scheme of the regeneration process of Tall coconut accessions by controlled pollination.

Characterization of the controlled pollination method

The manual pollinations were carried out according to the method described in details by De Nuce et al. (1980) and Yao et al. (2017). To characterize the method of regeneration of the collection, the

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Table 1. Tall coconut accessions regenerated between 2005 and 2010 in International Coconut Collection for Africa and the Indian Ocean (ICC-AIO) in Côte d'Ivoire.

No.	Accessions (population)	Codes	Year of introduction	Parental accession plot	Source (Region, Country)
1	West African Tall (Agriculture)	WAT	1952	091	Africa, Côte d'Ivoire
2	West African Tall (Akabo)	WAT3	1952	101	Africa, Côte d'Ivoire
3	West African Tall (Mensah)	WAT4	1954	142	Africa, Côte d'Ivoire
4	West African Tall (Ouidah)	WAT6	1952	M63	Africa, Benin
5	Cameroon Tall (Kribi)	CAT	1952	M63	Africa, Cameroun
6	Mozambique Tall (Companhia)	MZT	1970	M63	Africa, Mozambique
7	Polynesia Tall (Tahiti)	PYT1	1969	091	Pacific, Polynesia
8	New Guinea Tall (Kar-Kar)	NGT1	1975	102	Pacific, Papua New Guinea
9	New Guinea Tall (Markham Valley)	NGT4	1982	142	Pacific, Papua New Guinea
10	Solomons Tall	SNT	1982	091	Pacific, Solomon Islands
11	Indonesia Tall (Takome)	DOT1	1981	142	Far East, Cambodia
12	Indonesia Tall (Tenga)	DOT2	1982	142	Far East, Cambodia
13	Indonesia Tall (Palu)	DOT3	1982	142	Far East, Cambodia
14	Malayan Tall	MLT	1970	M63	Far East, Malaysia
15	Comoro Tall (Moheli)	COT	1972	111	Indian Ocean, Indian
16	Indian Tall (Andaman Ordinary)	NDT2	1968	142	Indian Ocean, Indian
17	Indian Tall (Andaman Géant)	NDT3	1968	142	Indian Ocean, Indian
18	Indian Tall (Kappadam)	NDT5	1968	142	Indian Ocean, Indian
19	Indian Tall (Laccadive Micro)	NDT7	1978	101	Indian Ocean, Indian
20	Indian Tall (Laccadive Ordinary)	NDT8	1979	101	Indian Ocean, Indian
21	Panama Tall (Aguadulce)	PAT1	1980	M63	Latin America, Panama
22	Panama Tall (Monagre)	PAT2	1980	M63	Latin America, Panama

Status	Vericp	Field	Row	Tree	Yplant	Mplant	Mother	Father	Plot	Access_nb	Num_feco	Num_noix	Mother_num	Mother_fie	Mother_row	Mother_tre	Mother_n
M		062	1	1	2010	5	GCA1	GCA1	1	PB3409	AN3417	4	P13389	M63	37	21	GCA1
		062	1	2	2010	5	GCA1	GCA1	1	PB3409	AN3482	3	P13373	M63	37	5	GCA1
M		062	1	3	2009	5	GCA1	GCA1	1	PB3409	AN3475	1	P13423	M63	39	6	GCA1
M		062	1	4	2010	5	GCA1	GCA1	1	PB3409	AN3482	8	P13373	M63	37	5	GCA1
		062	1	5	2009	5	GCA1	GCA1	1	PB3409	AN3239	2	P13355	M63	36	12	GCA1
		062	1	6	2009	5	GPY1	GPY1	2	PB3407	AN0992	3	P13185	091	28	19	GPY1
		062	1	7	2009	5	GPY1	GPY1	2	PB3407	AN1009	2	P13284	091	57	25	GPY1
		062	1	8	2009	5	GPY1	GPY1	2	PB3407	AN1009	1	P13284	091	57	25	GPY1
		062	1	9	2010	5	GPY1	GPY1	2	PB3407	AN3111	5	P13191	091	29	1	GPY1
		062	1	10	2009	5	GPY1	GPY1	2	PB3407	AN1270	4	P13296	091	60	24	GPY1
		062	1	11	2009	5	GND3	GND3	3	PB3408	AN3799	7	P13013	142	5	15	GND3
		062	1	12	2009	5	GND3	GND3	3	PB3408	AN4712	6	P13021	142	5	25	GND3
		062	1	13	2009	5	GND3	GND3	3	PB3408	AN4030	5	P12965	142	3	5	GND3
		062	1	14	2009	5	GND3	GND3	3	PB3408	AN3863	7	P13001	142	4	22	GND3
		062	1	15	2009	5	GND3	GND3	3	PB3408	AN1322	5	P12975	142	3	16	GND3
		062	1	16	2009	5	GQA3	GQA3	4	PB3401	AM7641	5	P12723	M63	22	8	GQA3
		062	1	17	2009	5	GQA3	GQA3	4	PB3401	AN0274	4	P12662	M63	18	5	GQA3
		062	1	18	2009	5	GQA3	GQA3	4	PB3401	AM7641	2	P12723	M63	22	8	GQA3
		062	1	19	2009	5	G					3	P12651	M63	17	12	GQA3
		062	1	20	2009	5	G					3	P12703	M63	21	2	GQA3
		062	1	21	2009	5	G					2	P08810	101	63	7	GND7
		062	1	22	2009	5	G					10	P12109	101	62	17	GND7
		062	1	23	2009	5	G					5	P08800	101	61	19	GND7
		062	1	24	2009	5	G					3	P12088	101	60	25	GND7
		062	1	25	2009	5	G					5	P08809	101	62	20	GND7
M		062	2	1	2010	5	GCA1	GCA1	1	PB3409	AN1768	2	P13324	M63	35	6	GCA1
		062	2	2	2009	5	GCA1	GCA1	1	PB3409	AN3207	1	P13353	M63	36	10	GCA1

Figure 1. Example of a Dbase file designed using Visual FoxPro software for recording the pedigrees of parental genotypes planted in the field.

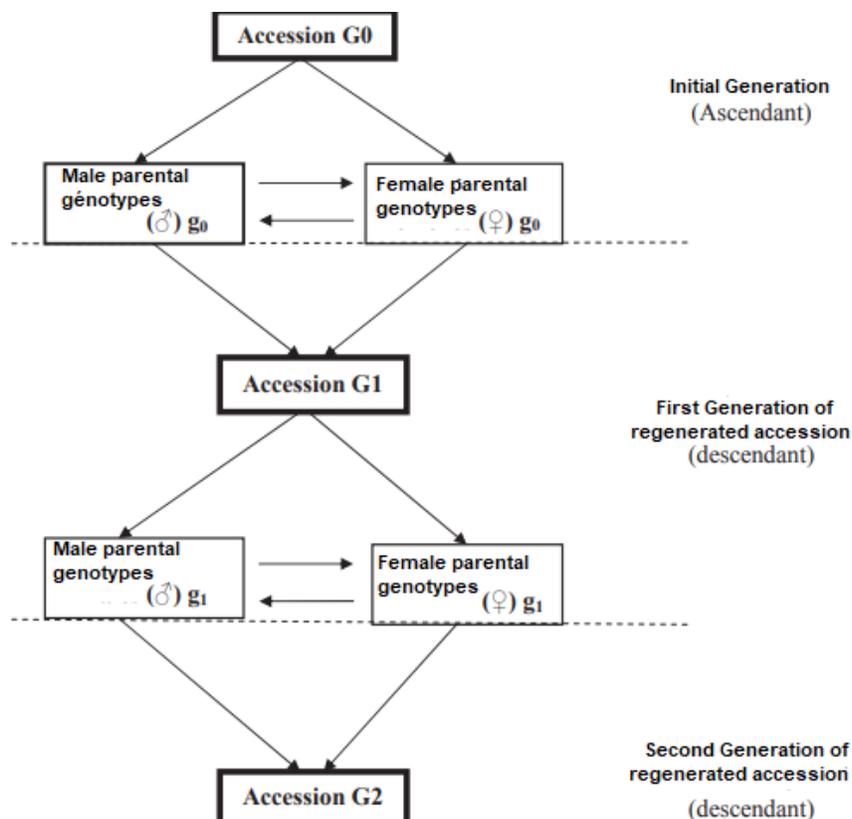


Figure 2. Scheme of the regeneration process of Tall coconut accessions by controlled pollination.

total number of pollinations carried out, the yield of plants planted from controlled fertilizations and the risk of errors were determined. The yield of controlled pollinations was assessed by the ratio between the total number of pollinations carried out and trees planted in the field. This index made it possible to roughly know the number of pollinations to be carried out per accession allowing planting a living progenies in the field. The proportion of individuals whose genealogy remained unknown or risk of error, after rigorous monitoring of the traceability of the regenerated plants planted in the collection from 2008 to 2011 was calculated.

Assessment of the representativeness of parental genotypes

Parental genotype representativeness variables

To assess the representativeness of the parental genotype used at each regeneration cycle, parental genotype representativeness variables were determined from the list of parental genotype previously drawn up. The number of parental genotypes was assessed by counting in each accession to be regenerated. Thus, based on the size of the initial accession and the number of parental genotypes used at the start of the regeneration program, the Selection Rates (ST), Representativeness (TR) and Representativeness Deviation (ER) of the parental genotype were calculated using the following formulas:

$$ST(\%) = \frac{\text{Number of parental genotypes used}}{\text{number of palm in initial accession}} \times 100$$

$$TS(\%) = \frac{\text{number of represented parental genotypes}}{\text{Size of parental genotypes used}} \times 100$$

$$ER(\%) = \frac{\text{number of parental genotypes used} - \text{number of parental genotypes represented}}{\text{number of parental genotypes used}} \times 100$$

The parental genotype selection rate (ST) measures the selection pressure of the parental genotype during their sampling in a given accession. In the current process of regeneration of the coconut palm collection in Côte d'Ivoire, the sampling of parental genotypes is carried out "blind", that is to say randomly. The representativeness rate (TR) indicates the proportion of parental genotypes used having given at least one offspring planted in the field. The representativeness gap (RE) quantifies the proportion of parents that were used but did not give progenies during the regeneration of a given accession.

Effect of progeny mortality, fertility and height of parental genotypes on their representativeness

Twenty-four months after planting, a survey of the mortality of young Tall coconut trees was made on the regenerated plots. Rates of seedling mortality and reduction in parental genotype representativeness were assessed.

To evaluate the fertility of the male parents involved in the crosses, three fertility variables were retained. These are the number of inflorescences produced per tree per year, the average mass of pollen produced per inflorescence and the average *in vitro*

germination rate of the pollen. The pollen is obtained by inflorescence according to the method described in details by De Nuce et al. (1980) and Yao et al. (2017). The *in vitro* pollen germination rate was determined using the method described by Yao et al. (2010). The averages of the mass of pollen produced by inflorescence and the rate of pollen germination were evaluated in the populations of male parents represented and not represented by studied accessions.

To assess the fertility of the female parents involved in the different crosses, three variables were selected. These are the average annual number of seeds produced naturally per parental genotype, the annual averages of seeds produced by controlled pollination and per tree and the average annual number of bunches produced per tree.

Stem height (HAUT) was measured in meters (m) from the base of the insertion point of the oldest leaf in the leaf crown to ground level using a measuring tape (NTC, 50 m) and a pole. To do this, the 0 m origin of the tape measure was firmly attached to the end of the pole, the length of which varies according to the individuals to be measured. Thus, all the individuals constituting the populations of represented and unrepresented parental genotypes were measured.

Diversity of parental genotype populations used at each regeneration cycle

The diversity of parental genotype populations used for the first regeneration cycle (g0) was monitored after two regeneration cycles. It was assessed through the parental genotype representativeness rate during successive regeneration cycles and the Simpson's diversity index. Simpson's diversity index (D) was calculated from the progeny of the first and second cycle of regeneration from each of the g0 parents. It measures the probability that two randomly selected genotypes in the first and second cycle of regenerated accession belong to the same parental and/or grandparent lineage. Its formula is as follows:

$$D = \frac{\sum_i^p N_i (N_i - 1)}{N(N - 1)}$$

With N_i the number of genotypes from the same parental lineage and N the total number of genotypes. The parental progeny considered in this study is a g0 progenitor sampled during the regeneration of the initial accession. To do this, the progeny of the g0 parents were followed within the regenerated populations of first and second cycle genotypes. Simpson's diversity index varies between 0 and 1. The closer it tends to 0, the lower this probability and the higher the diversity of regenerated progeny.

Methods of analyzing the data collected

The means and the Simpson's diversity index were calculated using Microsoft Office Excel 2007 software. Comparisons of the level of fertility and the height of represented and unrepresented parental genotypes were carried out using SPSS version 16.0 software using the Student test at the 5% level of significance.

RESULTS

Characterization of the controlled pollination method

On the plots for the conservation of genetic resources of Tall coconut palms regenerated between 2005 and 2010,

the palms in regenerated accessions have a number that has varied between 45 and 150 individuals with an average of 132 individuals. The chances of having at least one seedling planted by controlled pollination carried out in the NGT4, DOT1, DOT3 and DOT5 accessions are greater compared to those observed in the other Tall coconut accessions studied. It takes an average of 211 to 315 controlled pollinations to obtain respectively a regenerated accession of 100 to 150 individuals planted in the field. This generally corresponds to 2.1 n controlled pollinations to obtain a regenerated accession size of n individuals. Per accession, 5 controlled pollinations carried out made it possible to have at least 3 plants in the field. The proportions of individuals regenerated by accession whose pedigree is unknown vary between 0 and 5.13% with an average of 1.04% (Table 2).

Representativeness of parental genotypes

On sampled old Tall coconut germplasm conservation plots, on average, each parental accession was represented with a population of 142 individuals. For the regeneration of each of the old Tall coconut accessions, 66 parental genotypes were used on average, that is, a selection rate of 49.68%. The average numbers of female and male parental genotypes selected per accession were 58 and 32 respectively. Out of 100 parental genotypes used initially to regenerate a Tall coconut palm accession, 71 were represented or had at least one offspring planted in the field. An average of 29% of the parental genotype used initially did not represented in field-planted genitors. The differences in representativeness were evaluated between 38.19 and 27.97% respectively for female and male parents (Table 3).

Effect of progeny mortality rate on the representativeness of parental genotypes

Mortality rates of regenerating plants per accession after rigorous plant sanitary monitoring carried out from 2007 to 2010 varied from 0 to 34.78% (Table 4). For each accession of Tall coconut palms, out of a workforce of 100 regenerated plants planted in the field, a mean of 7 die. The representativeness of parental genotypes has fallen by an average of 1.56%.

Effect of the fertility of male and female parental genotypes on their representativeness

The level of fertility of the populations of male parents selected within the five Tall coconut palm accessions studied for their regeneration was similar for the represented and unrepresented parents (Table 5).

Table 2. Yields of planted plants from controlled pollination and risk of errors.

Codes	Number of seedlings planted	Total number of controlled pollinations carried out	Number of plants provided per controlled pollination carried out	Risk of errors (%)
WAT	117	380	0.31	3.42
WAT3	150	272	0.55	0.00
WAT4	150	216	0.69	0.00
WAT6	138	158	0.87	3.62
CAT	150	312	0.48	0.00
MZT	150	370	0.41	2.00
PYT1	150	351	0.43	0.00
NGT1	150	198	0.76	0.00
NGT4	192	136	1.41	0.00
SNT	117	356	0.33	5.13
DOT1	150	96	1.56	0.00
DOT2	150	546	0.27	2.00
DOT3	150	549	0.27	0.00
MLT	147	145	1.01	1.36
COT	150	763	0.20	0.67
NDT2	150	327	0.46	0.00
NDT3	150	213	0.70	0.00
NDT5	150	236	0.64	1.33
NDT7	150	91	1.65	0.00
NDT8	150	294	0.51	0.00
PAT1	139	365	0.38	1.44
PAT2	150	502	0.30	2.00
Minimum value	117	91	0.20	0.00
Mean	148	312	0.64	1.04
Standard deviation	13.97	166.57	0.42	1.46
Maximum value	192	763	1.65	5.13

Table 3. Representativeness of the parental genotypes used during the regeneration of old Tall coconut accessions from 2005 to 2010.

Codes accessions	Sizes of parental accessions	Number of parental genotypes sampled			Parental genotype selection rate (%)	Parental genotype representativeness rate (%)		
		Female	Male	Total		Female	Male	Total
WAT	158	81	36	91	57.59	56.79	77.78	73.63
WAT3	150	83	33	86	57.33	62.65	69.70	68.60
WAT4	150	54	30	57	38.00	55.56	80.00	70.18

Table 3. Contd.

WAT6	150	70	25	71	47.33	41.43	72.00	50.70
CAT1	150	66	28	76	50.67	56.06	75.00	60.53
MZT	148	42	26	44	29.73	52.38	88.46	61.36
PYT1	140	74	30	104	74.29	50.00	80.00	58.65
NGT1	40	36	26	39	97.50	61.11	88.46	82.05
NGT4	143	61	24	62	43.36	75.41	100.00	82.26
SNT	137	70	47	79	57.66	55.71	57.45	65.82
DOT1	48	20	9	22	45.83	75.00	88.89	86.36
DOT2	149	79	30	84	56.38	51.90	76.67	63.10
DOT3	147	52	33	59	40.14	59.62	75.76	66.10
MLT	150	33	37	47	31.33	75.76	59.46	82.98
COT	359	74	39	80	22.28	41.89	51.28	55.00
NDT2	149	65	35	75	50.34	72.31	71.43	68.00
NDT3	125	35	30	42	33.60	65.71	76.67	76.19
NDT5	100	53	32	62	62.00	66.04	71.88	69.35
NDT7	125	42	24	50	40.00	90.48	95.83	92.00
NDT8	125	55	39	61	48.80	80.00	58.97	77.05
PAT1	150	78	48	86	57.33	66.67	58.33	72.09
PAT2	150	64	33	70	46.67	81.25	81.82	84.29
Minimum value	40	20	9	22	22.28	17.24	51.28	50.70
Means	142.41	58.50	31.55	65.77	49.68	63.35	75.26	71.20
Standart deviation	57.64	17.77	8.27	19.89	16.31	12.87	12.78	10.85
Maximum value	359	83	47	104	97.50	90.48	100.00	92.00

Table 4. Variation in the representativeness rate of parental genotype in relation to the mortality rate of parental genotypes in the field.

Accession code	Number of seedlings planted	Number of dead plants	Plant mortality rate (%)	Variation in the representativeness rate of parental genotypes		
				Female	Male	Total
WAT	117	3	2.56	0.00	11.11	17.58
WAT3	150	5	3.33	0.00	0.00	0.00
WAT4	150	13	8.67	1.85	3.33	1.75
WAT6	138	48	34.78	7.14	0.00	4.23
CAT	150	10	6.67	0.00	0.00	0.00
MZT	150	0	0.00	0.00	0.00	0.00
PYT1	150	10	6.67	2.70	0.00	1.92

Table 4. Contd.

NGT1	150	5	3.33	0.00	0.00	0.00
NGT4	192	27	14.06	0.00	0.00	0.00
SNT	117	14	11.97	1.43	2.13	2.53
DOT1	150	4	2.67	0.00	0.00	0.00
DOT2	150	2	1.33	0.00	0.00	0.00
DOT3	150	2	1.33	0.00	0.00	0.00
MLT	147	5	3.40	0.00	0.00	0.00
COT	150	4	2.67	0.00	0.00	0.00
NDT2	150	6	4.00	0.00	0.00	0.00
NDT3	150	4	2.67	0.00	0.00	0.00
NDT5	150	6	4.00	0.00	0.00	0.00
NDT7	150	7	4.67	0.00	4.17	0.00
NDT8	150	2	1.33	0.00	0.00	0.00
PAT1	139	17	12.23	5.13	0.00	3.49
PAT2	150	18	12.00	3.13	0.00	2.86
Minimum value	117	2	0.00	0.00	0.00	0.00
Mean	147.72	9.63	6.56	0.97	0.94	1.56
Standart deviation	13.97	10.76	7.50	1.94	2.55	3.82
Maximum value	192	48	34.78	5.13	4.17	17.58

Table 5. Mean fertility variables of represented and unrepresented male parents in five Tall coconut accessions regenerated between 2005 and 2010.

Assession code	Parental genotypes	Average pollen germination rate (%)	Average mass of pollen (g) per inflorescence	Average annual number of inflorescences per tree
WAT	Represented	38.32 ± 2.67	9.25 ± 2.57	9.96 ± 1.67
	Not represented	37.62 ± 3.47	8.31 ± 3.06	11.20 ± 1.48
	p-value	0.343	0.182	0.115
	Represented	34.50 ± 3.24	7.72 ± 3.25	7.63 ± 2.56
PYT1	Not represented	31.80 ± 5.58	6.80 ± 3.89	6.50 ± 0.70
	p-value	0.156	0.583	0.550
	Represented	36.35 ± 2.43	6.92 ± 2.86	8.95 ± 2.70
SNT	Not represented	37.76 ± 2.84	6.71 ± 2.41	9.38 ± 1.66
	p-value	0.140	0.813	0.609
	Represented	36.50 ± 2.87	7.62 ± 9.29	12.26 ± 1.60

Table 5. Contd.

MZT	Not represented	39.50 ± 3.29	5.75 ± 2.21	10.66 ± 2.08
	p-value	0.106	0.286	0.128
	Represented	34.02 ± 2.41	8.50 ± 3.53	4.13 ± 2.22
NGT4	Not represented	39.02 ± 3.54	7.45 ± 3.73	6.5 ± 1.00
	p-value	0.096	0.700	0.062

p: Student's test probability value.

Concerning the female parents, the average number of seeds produced per tree and by controlled pollination was significantly different between the populations of represented and unrepresented parents. This number, which fluctuated between 2.07 and 3.25 for represented parents, was higher than that of unrepresented parents, evaluated between 0.67 and 1.28 (Table 6).

Effect of parental genotype height on their representativeness

Statistical analyzes differentiated represented and unrepresented parental genotypes for the stipe height descriptor. For all the Tall coconut tree accessions studied, the non-represented and represented parents were counted respectively among the tallest trees and those of small size (Table 7).

Diversity level of offspring from parental genotypes used at each regeneration cycle

The evolution of the representativeness of the parental genotype used initially for the regeneration of 3 accessions from the first cycle to the second cycle of regeneration is presented in

Figure 3. There is a reduction in the representativeness rate of the parental genotype during the successive cycles of regeneration. The parental genotype representativeness rate, which varied between 91 and 93% in the first regeneration cycle, dropped to between 51 and 62% in the second regeneration cycle.

The risk of encountering two parental genotypes belonging to the same grandparent lineage was higher in the second generation of regenerated accessions than in the first. This risk was evaluated between 3 and 7% in the second generation against 1 to 2% in the first generation of regenerated accessions. On average, the probability of encountering two parental genotypes belonging to the same grandparent progeny in the second generation of accession was 4 times higher than that recorded in the parental progeny (Figure 4).

DISCUSSION

To regenerate a Tall coconut accession with a number of progeny equal to n, it is generally necessary to carry out 2.1 n controlled pollinations. This result is in disagreement with the number of pollinations advocated by Wang et al. (2004) for plant regeneration in general. These authors stipulate that to regenerate a plant

accession of size n, the realization of 0.5 n crosses are necessary. This difference in the number of crossings to be carried out could be explained by the particular nature of the coconut palm whose seed yields from controlled pollination are low. The low yields of controlled pollination can be attributed to the quality of the pollen, the depressive effect of the inflorescence isolation bag on the fertility of the mother tree and the type of regenerated coconut tree (Yoboue, 2009; Oyiga et al., 2010; Yao et al., 2010).

The number of seedlings provided per controlled pollination carried out varies from one Tall coconut accession to another. The variability in the reproductive performance of coconut palm accessions would be of a genetic nature given that they come from various geographical areas and are installed in the same environmental and experimental conditions of the Marc Delorme Station. Such an argument has been made by Guetet et al. (2008) to differentiate natural populations of *Allium roseum* in Tunisia according to their reproductive vigor. The proportions of individuals regenerated by accession whose pedigree is unknown vary between 0 and 34.78% with an average of 1.56%. These genotypes of unknown genealogy should be replaced because they could increase the proportion of illegitimate (Bourdeix et al., 2010). On the plots for the conservation of genetic resources of Tall coconut

Table 6. Average of fertility variables of represented and unrepresented female parental genotypes in five Tall coconut accessions regenerated between 2005 and 2010.

Accession code	Parental genotypes	Average number of seeds produced per tree and per controlled pollination carried out	Annual average number of seeds produced in natural conditions per tree	Average annual number of bunches per tree
WAT	Represented	2.07 ± 1.34	59.53 ± 21.19	10.15 ± 1.53
	Not represented	1.28 ± 1.23	54.96 ± 18.28	9.93 ± 1.86
	p-value	0.013	0.340	0.593
	Represented	2.07 ± 1.07	37.46 ± 22.29	7.73 ± 2.70
PYT1	Not represented	0.67 ± 1.29	43.38 ± 29.23	8.55 ± 2.71
	p-value	<0.001	0.366	0.225
	Represented	2.25 ± 1.25	64.62 ± 39.89	9.85 ± 2.69
SNT	Not represented	1.13 ± 0.94	52.45 ± 26.61	9.18 ± 2.50
	p-value	0.001	0.227	0.376
	Represented	3.25 ± 1.06	67.38 ± 33.66	11.76 ± 1.99
MZT	Not represented	2.30 ± 1.71	60.60 ± 27.22	11.75 ± 1.94
	p-value	0.044	0.484	0.985
	Represented	2.09 ± 1.55	31.75 ± 22.37	5.33 ± 2.46
NGT4	Not represented	1.05 ± 1.58	25.72 ± 13.71	5.09 ± 1.79
	p-value	0.032	0.261	0.690

p: Student's test probability value.

Table 7. Influence of parental genotype height on their representativeness.

Accession code		Parental genotype not represented	Parental genotype represented
WAT	Means	12 ± 0.43	9.37 ± 0.88
	p		< 0.001
PYT1	Means	10.38 ± 0.95	9.33 ± 1.52
	p		0.01
SNT	Means	12.33 ± 0.65	11.27 ± 1.51
	p		0.02
MZT	Means	12.11 ± 0.48	10.93 ± 0.99
	p		< 0.001
NGT4	Means	11.9 ± 0.73	9.94 ± 1.24
	p		< 0.001

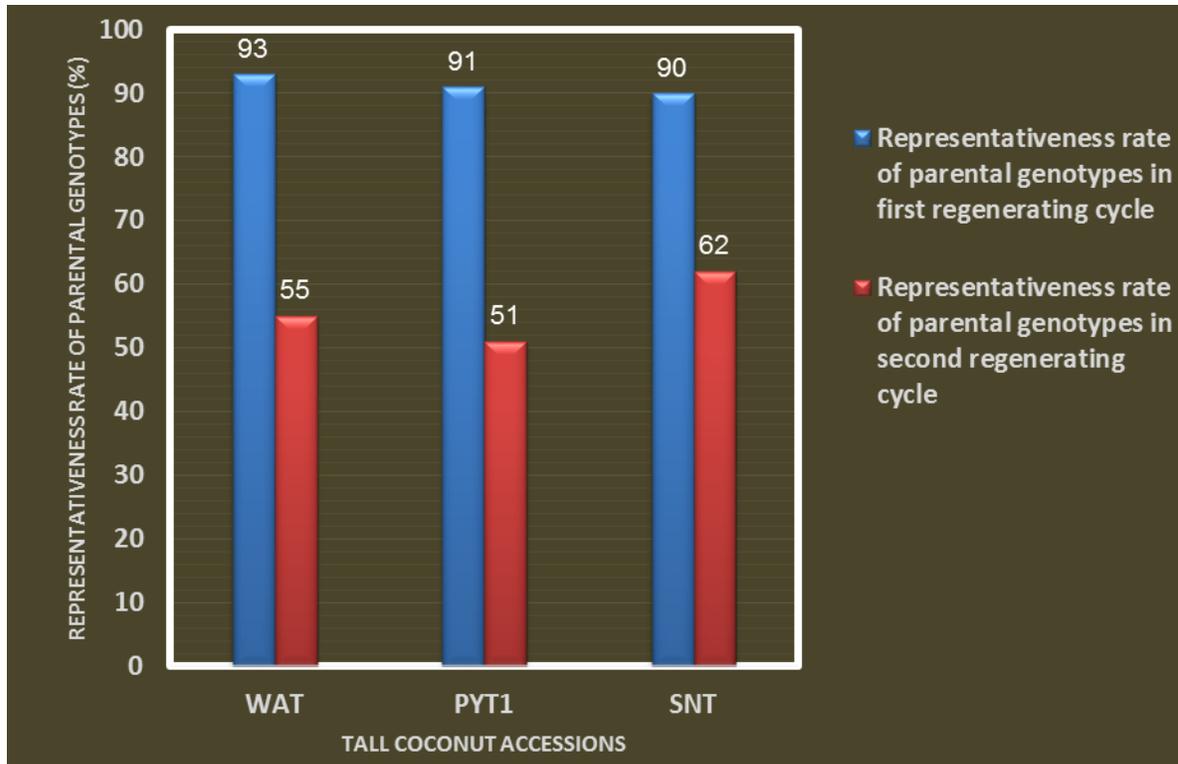


Figure 3. Evolution of the representativeness rate of the parents used for the regeneration of three accessions of Tall coconut palms from the first to the second generation.

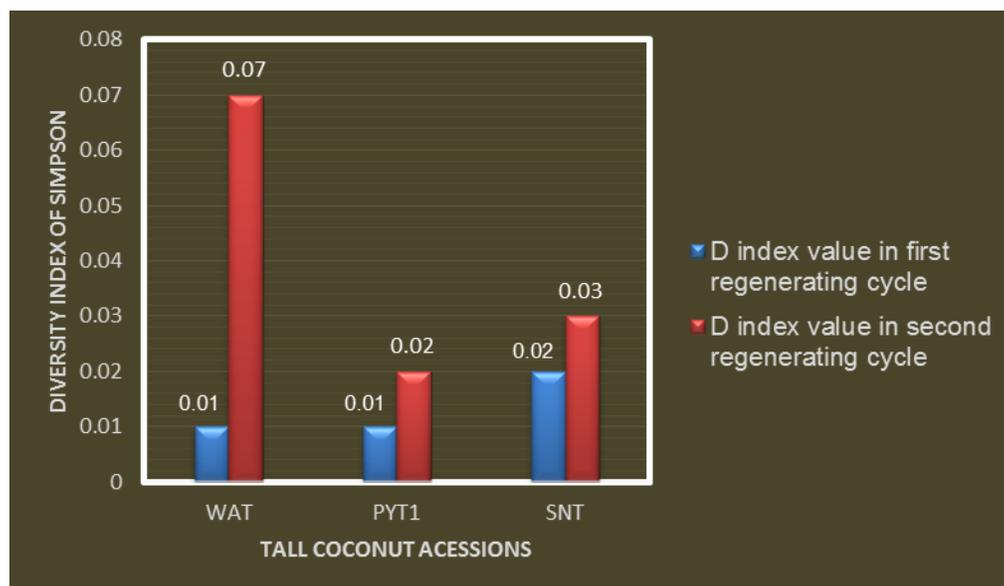


Figure 4. Evolution of the diversity index of Simpson of the progeny of parental genotypes in the first and second cycle of regenerated population in three Tall coconut accessions.

palms regenerated between 2005 and 2010, the regenerated accessions have a number that varies

between 117 and 192 individuals with an average of 148 individuals. When the average mortality rate of 6.56% is

taken into account, this average is reduced to 138 live trees in the field. This average of parental genotypes conserved in the field seems acceptable insofar as on the old conservation plots of Tall coconut palm genetic resources, on average, each parental accession is represented with a population of 142 individuals. This result would reflect a conservation of the richness in genotypes of the regenerated accessions of the international coconut palm collection in Côte d'Ivoire (Bourdeix, 1989).

Indeed, regenerated Tall coconut accessions are represented by at least one hundred genotypes. For these young and old coconut genetic resource conservation plots, this high number of genotypes present would reflect the low mortality of individuals in the field. This maintenance of a sufficient level of genotype richness in the field could be explained by the fact that Tall coconut palms are very resistant to diseases, pests and drought (Konan et al., 2006).

These causes of mortality constitute the main potential sources of loss of genotypes and therefore of diversity in *ex situ* collections of perennial plants (Mohd and Ramanatha, 2001). Similarly, these results testify to the good phytosanitary monitoring of accession conservation plots at the Marc Delorme Station. This preserves the genotypes in the field against the enemies of the coconut palm (Allou and De Franqueville, 2001; Allou et al., 2006; N'Goran et al., 2009). Also, it should be added that the favorable geographical location of the Marc Delorme Station avoids the uprooting of genotypes preserved by high winds and cyclones compared to countries such as Nicaragua, Malaysia and the Philippines.

For the regeneration of each of the old Tall coconut accessions, 66 parental genotypes are sampled on average, i.e. a selection rate of 49.68%. The average numbers of male and female parental genotypes selected per accession are 32 and 58 respectively. The lower number of male genitors is explained by the fact that pollen is collected from male flowers, which are more abundant on the inflorescence (Konan et al., 2008). The number of genitors indicated is sufficient to provide the quantity of pollen required for regeneration. The number of female parental genotypes is more because it is the male flowers that are castrated. Unfortunately, female flowers are limited in number (20 to 30 units) in Tall coconut trees. The number of parental genotypes is close to that recommended by Bourdeix et al. (2010) of 25 to 50 male genitors for 70 to 100 females.

An average of 29% of initially sampled parental genotype did not yield field-planted regenerators. The differences in the representativeness of the parental genotypes highlighted in the current regeneration process would be due to the random sampling of seeds of regenerators on the parental genotypes without taking their genealogy into account (Crossa and Vencovsky, 1997; Vencovsky and Crossa, 2003). When looking specifically at female and male parental genotypes, only

63.35% and 75.26% gave field-planted offspring, respectively. From these results, it appears that the female parents are less represented than the male parents during the process of regeneration of the large coconut collection in Côte d'Ivoire. This could be explained by the fact that the pollen from the same male parent is used in the cross with several mother plants. Such an observation goes against the recommendations resulting from the work of Vencovsky and Crossa (1999). These authors reported that it is necessary to favor the representativeness of the mother plants during regeneration for a satisfactory maintenance of the genetic integrity of the accession to be regenerated. Indeed, female gametic control in seed collection ensures that at least half of the genes transmitted to the next generation come from the initial accession. Such a seed collection strategy would be suitable for Tall coconut palms insofar as the control of the origin of the pollen does not exclude the risk of contamination (De Nuce et al., 1980).

Considering the importance of this collecting strategy in the conservation of allogamous plant genetic resources, Johnson et al. (2004) recommend treating at least three inflorescences on the same mother plant to ensure its representativeness during the regeneration process. According to Vencovsky and Crossa (2003), female gametic control makes it possible to recover a representativeness rate of female parents of around 20%.

In addition, the results showed that except for the average number of seeds per tree by controlled fertilization, the level of fertility of male and female parents, represented and unrepresented, selected within each of the parental accessions is similar. This suggests that differences in representativeness of parental genotypes are not caused by the amount of pollen produced, the average number of inflorescences and bunches produced per tree, and the average number of seeds obtained by open fertilization in male and female parental genotypes. Indeed, the uniform expression of these characters listed above, by the parents represented and not represented, would find its origins in the allogamous nature of the coconut tree. Indeed, this mode of reproduction favors a very great variability in the expression of characters in the genotypes within the same accession of Tall coconut palm. This diversification of the constituent genotypes of Tall coconut palm accessions was reported by Konan et al. (2007). Similarly, chance in the distribution of pollen from male parental genotypes would favor the abusive use of certain trees as parental genotypes during regeneration.

The study revealed that the mortality rate of the parental genotypes planted in the field, the height of the parental genotypes and the average number of seeds per tree by controlled pollination would be at the origin of the differences in the representativeness of the parental genotypes used during the regeneration in the coconut palm. Regarding the mortality rate of parental genotypes in the field, a rate of 6.56% is considered low. This author

stipulates that at the end of the regeneration process of a coconut palm accession, the field mortality rate of the plants can be estimated at 5%.

The consideration of this mortality threshold as an index of the smooth running of a coconut tree regeneration operation can be validated from the present study in the sense that it has a low impact on the representativeness of the parental genotypes. Indeed, with 5.36% mortality of regenerators in the field, the representativeness rate of parental genotypes is reduced by only 1.04%.

Concerning the height of parental genotypes in the field, those of small size are improperly sampled. This would be explained by the ease of access to the leaf crown of the tree by the technicians and the reduction of the risk of accidents. Consequently, the unrepresented parents within the regenerated accessions are counted among the excessively tall trees. The populations of parental genotypes represented are characterized for their part by an average height different from that of the population from which they come. This deviation varies from 6 to 13%. The decrease in phenotypic groups in the represented parent population compared to those in the parent population could affect the distribution of genes across generations. This would increase the risk of genetic drift in the sense that the large size of certain trees in the field reduces the participation of all the parents selected in the regeneration of the next generation of accession (Vencovsky and Crossa, 1999).

Regarding the yields of controlled fertilization, the results revealed the existence of variability in the reproductive performance of female parents in seed production mode by controlled pollination within coconut palm accessions. These results would mean that the parents who give more seeds during their reproduction by the controlled pollination method are very likely to have descendants in the next generation. Indeed, the non-representativeness of certain parents during their regeneration results from their unequal contribution in seeds for the constitution of the next generation. This would affect the distribution of genes through regeneration cycles. Similar results were reported by El-Kassaby et al. (2010).

In order to propose a strategy for efficient exploitation of parental genotypes in a new regeneration cycle, the monitoring of the representativeness of parental genotypes through two regeneration cycles was evaluated. The results first revealed a reduction in the representativeness of grandparents within populations of regenerated second-generation accessions. Three hypotheses would be more likely to explain these results. First, the loss of representativeness would be due to the elimination of certain parental genotypes in the regeneration process because of their excessive height. Then, the low seed yield of controlled pollination in some parents would lead to their extinction during the regeneration cycles. Finally, it will be necessary to add, to a lesser extent, the mortality of offspring in the field.

These results are consistent with those relating to the extinction of parental genotypes through generations of regeneration. Indeed, the higher the frequency of appearance of the parental genotype in cycle n , the lower the risks of its extinction in cycle $n+1$. Simpson's diversity indices are on average 0.04 in the second generation against 0.01 in the first generation. This result reflects a reduction in the state of diversity of the descendants of parent populations in the $n+1$ cycle. This could lead to greater genetic resemblance between individuals and inbreeding as mentioned by Noryazdan (2009) in sunflower.

Conclusion

The study made it possible to specify that to regenerate a Tall coconut accessions with n individuals, $2.11 n$ controlled pollinations must be carried out. In addition, the analysis highlighted differences in the representativeness of the parental genotypes sampled during the regeneration process of a Tall coconut accessions by controlled pollinations. These differences in the representativeness of the parental genotypes are caused by the mortality in the field of the regenerated plants planted, the excessive height of certain parental genotypes as well as their low seed yield by controlled pollinations. The evolution of the diversity level of the progenies from the parents used at each regeneration cycle showed a reduction in their genetic base during the successive regeneration cycles. Thus, in future programs to regenerate coconut accessions (Tall or Dwarf ecotypes), it is essential to take their pedigree into account when sampling genitors so as not to reduce the genetic base in regenerated populations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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