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Statistical models for coconut (*Cocos nucifera* L.) pollen fertility prediction according to its age and biochemical composition

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Abstract

This investigation was conducted to propose predicting models of stored pollen fertility through simple linear regression equations to improve the yields of coconut (*Cocos nucifera* L.) seeds produced by controlled pollination method. Therefore, pollen samples extracted from both coconut populations Comoro Moheli Tall (CMT) and Malayan Yellow Dwarf (MYD) planted in field coconut genebank at Port-Bouët, Côte d'Ivoire, were used. Therefore, 2 pollen fertility components, fruit-set rate and *in vitro* pollen germination rate and 5 biochemical traits that are moisture, lipid, protein, total sugars and reducing sugars contents in pollen extracted initially (0 month) then conditioned vacuum and stored in deep-freezer at -15°C to reach the pollen ages that are 1, 2, 3 and 4 months were studied. The results showed that *in vitro* pollen germination rate assessed at the laboratory was the best indicator having explained 64% fluctuations of fruit-set rate at the field in Tall coconut ecotype. Rates of *in vitro* pollen germination were predicted in 56.25% of the cases by its total sugars content and in 79.21% by pollen age respectively in Tall and Dwarf coconut palms studied. Mathematical models relatively of the fruit-set and *in vitro* pollen germination rates revealed in this study could be helpful to an efficient management of pollen bank for increase seed production by hand pollinations in coconut populations.

Keywords: coconut pollen, pollen fertility, biochemical compounds, linear regression models, controlled pollination, Côte d'Ivoire

1. Introduction

The coconut palm (*Cocos nucifera* L., Arecaceae) is a perennial monocotyledon from two geographical origins, Pacific and Indo-Atlantic oceanic basins (Gunn *et al.*, 2009) [1]. This plant is monoïque, diploid (2n = 2x = 32 chromosomes) and an oil crop which plays an important role in households all over the tropical world (Lebrun *et al.*, 2001) [2]. Within the genus *Cocos* where *Cocos nucifera* L. is the sole species, two main groups are identified, the Tall and Dwarf palms which are cross-fertilized and self-fertilized respectively. In the axil of each leaf originate from the growing point at the apex of the stem there is a floral bud that will evolve or no in inflorescence (Yao, 2008) [3]. Before opening, inflorescence has spear-like shape enclosed within in sheath designed spathe. At mature stage, the spathe cracks longitudinally and let appear the inflorescence that is a spadix. The spadix consist of a central axe with many spikelets that carry in their basal part female flowers surmounted of male flowers that give all at mature stage a viable pollen. Pollen or male gamete produced from diploid cells following meiosis and mitosis successions is haploid (Yao, 2008) [3]. The coconut pollen grain is binucleate with 30 µm of diameter (Santos & Emmanuel, 2004) [4].

To our knowledge, in coconut improvement programs of several countries, data about predicting models of seed yields from hand pollinations realized at the field according to the pollen viability assessed at the laboratory are not sufficiently well stocked. The coconut breeding strategies used in Côte d'Ivoire are based on the conservation and assessment of accessions in the coconut genebank, the germplasm use for detecting the best combinations among ecotypes and the improvement through development of hybrids identified through performance tests among progenies (Gascon & De Nuce, 1976) [5]. This scheme has been slightly restructured into different axes (Tall x Tall, Dwarf x Tall and Dwarf x Dwarf) using the Reciprocal Recurrent Selection method (Bourdeix *et al.*, 1990) [6]. To supply the research activities specially hand pollinations at the field good quality of pollen stored at cold is require. This pollen bank is localized currently at the Centre National de Recherche Agronomique (CNRA) of Marc Delorme Research Station.

In general, for seed productions, conserved pollen viability seem influenced the success of the hand pollinations as reported by Charier (1990) [7] and Colas & Mercier (2000) [8]. At Côte d'Ivoire for coconut seed productions, results about importance of the storage of freeze-dried pollen (Bernard, 1973) [9], cold storage of pollen (Rognon & De Nuce, 1973) [10], composition of the medium for *in vitro* pollen germination test (Verdel & Pannetier, 1990) [11], the depressive effect of bagging used in inflorescence isolation (Yoboue, 2009) [12], the influence of pollen quality on seed yields (Yao *et al.*, 2010) [13] and controlled pollination protocol (Konan *et al.*, 2008) [14] are known. Besides, Yao *et al.* (2010) [13] revealed that the Tall and Dwarf coconut pollen extracted initially and stored in deep-freezer differ in their macronutrient contents. For modeling seed yields coming to maturity in Tall x Tall coconut hybrids from the fruit-set Issali *et al.* (2013) [15] were reported in Côte d'Ivoire that 83.70% fructification variations were due to fruit-set.

However, there are scanty reports about modeling approaches of fruit-set and *in vitro* pollen germination rates according to stored pollen age and its macronutrient contents. The current study while studying biochemical modifications during pollen storage and their effects on its fertility aims to provide some mathematical models for efficient stored pollen using in the conditions of Marc Delorme Research Station of Côte d'Ivoire and to enhance coconut breeding success.

2. Materials and methods

The experiments were conducted at the Marc Delorme Coconut Research Station (N 5°14.5' - W 3°54.5') at Port-Bouët, Southern Côte d'Ivoire. For modeling fruit-set and pollen germination rates, pollen samples extracted in male flowers harvested from two coconut populations that are Comoro Moheli Tall (CMT) and Malayan Yellow Dwarf (MYD) were used. Pollen grains were extracted initially from male flowers dried at 40°C in oven after 20 h then conditioned vacuum and stored in deep-freezer at -15°C during 1, 2, 3 and 4 months. The sampling, conditioning and storage of pollen relating to these two coconut populations were described in detail in previously paper published by Yao *et al.* (2010) [13]. Details about operations led during controlled pollination were showed in Fig. 1.

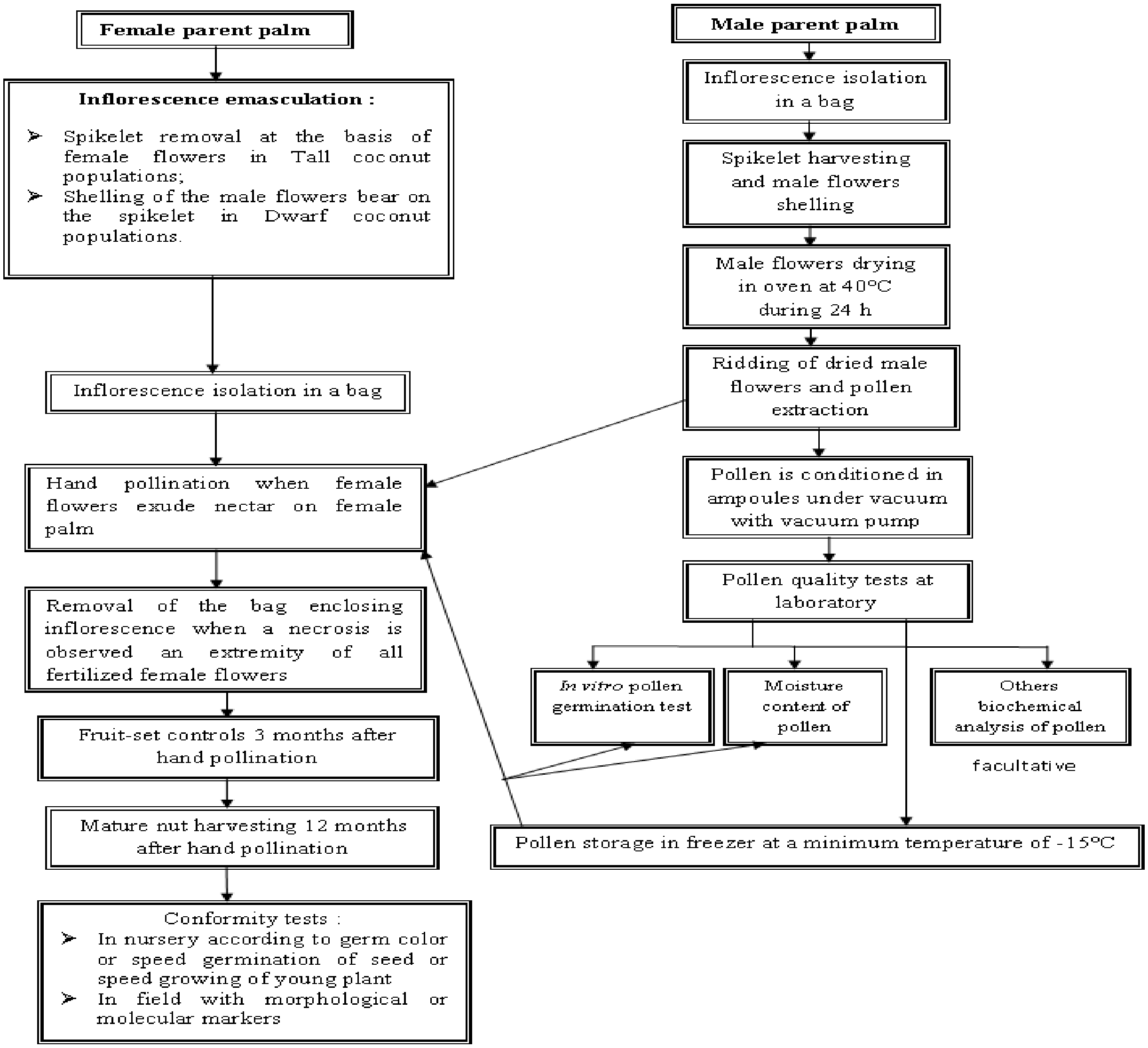

Fig 1: Controlled pollination process used at the Marc Delorme Research Station in Côte d'Ivoire

Morphology through the color and grain aspects of coconut pollen extracted immediately from dry male flowers was observed and described. Pollen germination was done from 0.25 g of pollen grains in Petri dish on solid medium containing 1.2 g of agar-agar, 11 g of sucrose and 100 ml of distilled water (De Nuce *et al.*, 1980) [16]. After 3 h of incubation, the morphology of pollen grains in germination onto this solid medium was also observed under Light Microscope (LM) equipped of a camera VC 3011 at 100x magnification. Then, the shapes of pollen grains, pollen tube lengths, density of intra-cellular of germinated and non-geminated pollen grains were observed. Pollen germination rate (PGR) was calculated as described by Colas & Mercier (2000) [8] and De Nuce *et al.* (1980) [16]. Pollen moisture content was measured by the high-temperature oven method: 105°C for 24 h (De Nuce *et al.*, 1980) [16]. Lipids fractions were extracted from 3.5 g of dry pollen sample from Soxhlet method with hexane as solvent according to an ISO 659 norm (AFNOR, 1986) [17]. Proteins, total sugars and reducing sugars contents were determined on removed oil of dry pollen samples following the standard methods of Association of Official Analytical Chemists (AOAC, 1980) [18].

In the field, the crosses were done according to controlled pollination method as showed on Fig. 1. To minimize effect of pollen competition healthy male and female parent palms were selected randomly among the same coconut population. Within each coconut population studied cross-pollinated were done between chosen pollinators and emasculated female palms without reciprocal and self-pollinations. Three months (90 days) after crossings, fruit-set numbers per bunch were counted and the fruit-set rates (FSR) were calculated from initial numbers of female flower per inflorescence. During 5 months, i.e. from December 2006 to April 2007 monthly means of female flowers number produced per inflorescence varied from 23 to 24 in CMT and MYD respectively. During periods covering hand pollination (December 2006 to April 2007) and fruit-set assessment (March 2007 to July 2007) monthly averages of rainfall, temperature and humidity were 131.08 mm, 26.71°C and 88.33% respectively.

The statistical analyses were performed in 2 steps using SPSS v. 16.0 (SPSS Inc., Chicago, USA) software. The first step consisted to search variables affected significantly during pollen storage. Thus, one-way ANOVA with only factor "pollen age" was achieved. The variables which the probability associated to ANOVA test was significant at 5% threshold in both coconut populations CMT and MYD were retained for the second step. The interrelationships between these variables were calculated from Pearson’s correlation and facilitated the determination of the ones to consider for the stepwise linear regression analysis. Then, to identify the best model of linear regressions the contribution of the pollen age and biochemical parameters in pollen fertility fluctuations (Pollen germination and Fruit-set rates) through the significance of the regression model and the fitness quality R^2 were assessed. The following regression model was tested:

$$Y = a + b_1 * X_1 + b_2 * X_2 + \cdots \ldots\ldots\ldots + b_i * X_i \qquad \textbf{(1)}$$

where “Y” is the dependant variable that represents FSR or PGR, “X_i” the independent variable that is biochemical variables or PGR, “a” the intercept and “b_i” the partial regression coefficient that specify the empirical relationships between Y and X_i.

3. Results

3.1 Coconut pollen morphology

Whatever coconut population, Tall or Dwarf, the pollen extracted from dried male flowers was powdery with a yellowish color (Fig. 2). Coconut pollen grains under Light Microscope (LM) at 100x magnification have an oval shape as shown in Fig. 3. Within the same pollen sample during *in vitro* germination onto solid medium there are some pollen grains with various pollen tube lengths (germinated pollen grains) and others that are no pollen tube (non-germinated pollen grains). Cytoplasm of pollen grains in germination was less dense than those that didn't germinate as shown in Fig. 3.

Fig 2: Macroscopic observation of coconut pollen sample extracted from males flowers dried at 40°C in oven after 20 h

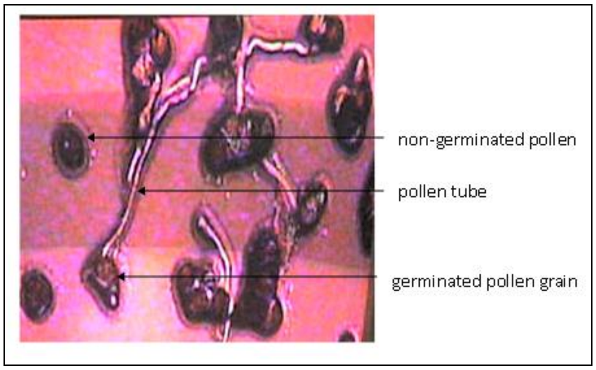

Fig 3: Coconut pollen grains in germination on solid medium under Light Microscope (LM) at 100x magnification

3.2 Effect of pollen age on its fertility and biochemical compositions

Fruit-set rate decreases when pollen age increases (Fig. 4-5). In Table 1, whereas fruit-set rate varied significantly during pollen storage for CMT (F = 3.83; p = 0.039) there is no significant for MYD (F=0.96; p=0.467). The rates of *in vitro* pollen germination were significantly affected by pollen age increase in CMT (F = 11.79; p = 0.001) and MYD (F = 11.77; p = 0.001) coconut populations as shown in Table 1. In Table 1, proteins, total sugars and reducing sugars contents were also influenced by conserved pollen age increase in both two coconut populations studied.

Meanwhile, the two biochemical parameters that are lipids and moisture contents which remain constant in pollen during its storage were eliminated for the survey continuation (Table 1).

Fig 4: Efficient fruit-set per bunch three months after hand pollination in coconut achieved with 1 month of stored pollen age

Fig 5: Low fruit-set per bunch three months after hand pollination in coconut achieved with 4 months of stored pollen age

Table 1: Effect of stored pollen age (from 0 to 4 mouths) on its fertility and biochemical components in two coconut populations, Comoro Moheli Tall (CMT) and Malayan Yellow Dwarf (MYD)

Group of traits studied (Unit)		Ecotype	Min - Max	ANOVA test		
				MS	F	p
Pollen fertility components (%)	FSR	CMT	1.2-20.5	217.47	3.83	0.039
		MYD	1.1-14.2	76.13	0.96	0.467
	PGR	CMT	27.6-39.8	71.84	11.77	0.001
		MYD	29.7- 40.1	11.19	11.79	0.001
Biochemical compositions (g/100 g of pollen extracted from dried male flowers)	LIP	CMT	9.1-10.6	1.03	1.02	0.440
		MYD	8.3-9.6	1.06	1.06	0.422
	MC	CMT	9.4-11.5	2.26	0.49	0.738
		MYD	7.8-10.9	0.52	0.52	0.718
	PROT	CMT	7.1-29.8	364.53	13.58	<0.001
		MYD	8-30	316.38	46.14	<0.001
	TS	CMT	6.4-9	3	10.15	0.002
		MYD	3.3-6.5	6.85	4.49	0.045
	RS	CMT	0.5-1.1	0.17	4.97	0.018
		MYD	0.4-1	0.17	4.09	0.032

FSR : Fruit-set rate ; PGR : *in vitro* pollen germination rate ; LIP: lipids content ; MC: Moisture content ;.PROT: Proteins content ; TS : Total sugars content ; RS : Reducing sugars content; Min: minimum value; Max: maximum value; df: degree of freedom ; MS: Means of square; p : probability value associated to ANOVA test

3.3 Mathematical models of fruit-set and in vitro pollen germination rates

3.3.1 Mathematical model of fruit-set rate

The rate of fruit-set was linked negatively with pollen age ($p = 0.015$; $r_{FSR/PA} = -0.61$), positively with the rate of *in vitro* pollen germination ($p < 0.001$; $r_{FSR/PGR} = 0.80$) and total sugars rate ($p = 0.021$; $r_{FSR/TS} = 0.59$) in Tall coconut population CMT as shown in Table 2. When the three parameters that are pollen age, pollen germination rate and total sugars content were entered at the same time for developing the multiple or simple regression models from stepwise method pollen germination rate was only retain in the model Eq. (2). So, the simple regression model explaining 64% of FSR fluctuations measured at the field was established following the relation (Table 3):

$$FSR_{/CMT} = 1.5 * PGR - 43.7 \qquad (2)$$

3.3.2 Mathematical models of in vitro pollen germination rate

Pollen germination rate (PGR) was correlated positively to proteins content ($p = 0.012$; $r_{PGR/PROT} = 0.63$) and total sugars content ($p = 0.001$; $r_{PGR/TS} = 0.75$) and negatively to pollen age ($p = 0.002$; $r_{PGR/PA} = -0.74$) in Tall coconut population CMT as shown in Table 2. For Dwarf coconut population MYD the rate of pollen germination was associated significantly to pollen age and proteins content where Pearson's correlation values were – 0.89 and 0.79 respectively at $p < 0.001$ (Table 2). For developing multiple or simple regression models only total sugars content and pollen age as the best parameters were implied in the explanation of PGR variations for CMT in Eq. (3) and MYD in Eq. (4) respectively. The equations of simple regression models explaining more than 50% of the PGR fluctuations at the laboratory were the following relations (Table 3):

$$PGR_{/CMT} = 3.6 * TS + 5.1 \quad (3)$$

$$PGR_{/MYD} = -2.6 * PA + 39.3 \quad (4)$$

Table 2: Relationships among six traits varying significantly during pollen storage in two coconut populations, Comoro Moheli Tall or CMT (above diagonal) and Malayan Yellow Dwarf or MYD (below diagonal)

CMT MYD	PA	FSR	PGR	Prot	TS	RS
PA	-	-0.61*	-0.74**	-0.88***	-0.83***	0.39
FSR	-0.52*	-	0.80***	0.50*	0.59*	0.31
PGR	-0.89***	0.66**	-	0.63*	0.75**	0.12
Prot	-0.93***	0.48	0.79***	-	0.71**	-0.36
TS	-0.61*	0.18	0.28	0.61*	-	-0.12
RS	0.40	0.35	-0.37	-0.49	-0.18	-

PA : Pollen age ; FSR : Fruit-set rate ; PGR : *in vitro* pollen germination rate ; Prot: Protein content ; TS : Total sugars content ; RS : Reducing sugars content ; p : Value of probability of Pearson's correlation test; *Significant Pearson's correlation at $p < 0.05$; **Significant Pearson's correlation at $p < 0.01$; ***Significant Pearson's correlation at $p < 0.001$

Table 3: Mathematical models to predict fruit-set and in vitro pollen germination rates in two coconut populations, Comoro Moheli Tall (CMT) and Malayan Yellow Dwarf (MYD)

Simple regression models	R^2 (%)	ANOVA tests on the linearity			Tests on the intercept (a) and partial regression coefficient (b)	
		df	F	p	Coefficients	p
$FSR_{/CMT} = 1.5*PGR – 43.7$	64.00	1	24.32	<0.001	a	<0.001
					b	0.001
$PGR_{/CMT} = 3.6*TS + 5.1$	56.25	1	15.69	0.002	a	0.002
					b	0.003
$PGR_{/MYD} = -2.6*PA + 39.3$	79.21	1	22.57	<0.001	a	<0.001
					b	<0.001

R^2: fitness quality; df: degree of freedom; p: value of probability; F: statistical value associated to ANOVA test; PA: pollen age; FSR: fruit-set rate; PGR: *in vitro* pollen germination rate; TS: total sugars content; a: intercept; b: partial regression coefficient

4. Discussion

Under Light Microscope (LM) at 100x magnification some morphological characteristics of coconut pollen were observed. The results were showed that coconut pollen grains have an oval shape. Oval shapes of pollen grains were reported in *Dacryodes edulis* (Don) Lam. (Yombi *et al.*, 1998) [19], that is an oleaginous growing in humid equatorial area of Africa and produces binucleate (Makueti *et al.*, 2012) [20] as reported in coconut palm (Santos & Emmanuel, 2004) [4]. Nevertheless, further studies should be achieved to refine the results about coconut pollen morphology. Indeed, Yombi *et al.* (1998) [19] were showed that pollen morphology affects its viability during storage. In this respect, further studies must be conducted to assess polar diameter-equatorial diameter ratio, the structure of the exine and the number of the apertures as achieved by Yombi *et al.* (1998) [19]. Already, we think that external membrane of coconut pollen is thick because its binucleate nature has been mentioned (Santos & Emmanuel, 2004) [4]. Indeed, Bajaj (1987) [21] reported that the binucleate pollen grains are scarcely affected by desiccation because their endowed thicker exine. Likewise, the pollen of coconut palms tolerates a strong reduction of its water content until 4% (Charier, 1990) [7] or between 6-8% (Verdel & Pannetier, 1990) [11] what reduces its breathing during storage. Therefore, later works in relation to the coconut pollen morphology should be described from standard terminologies (Walker & Doyle, 1975) [22]. Some standard terminologies of pollen morphology studied in Rosaceae (Tahir, 2005) [23] and Acanthaceae (Ghosh & Karmakar, 2012) [24] plant families from methods of Light Microscope (LM) and Scanning Electron Microscope (SEM) concern its polarity, symmetry, shape, type/aperture, sculpture, exine thickness, length and width of the aperture and aperture shape. Within a same sample germinated coconut pollen grains on solid medium have the various tube lengths. This result suggests that within a same sample, coconut pollen grains don't have the same vigor, i.e. necessary time able to permit pollen tubes to reach ovule and to achieve fertilization (Shivanna & Cresti, 1989) [25]. Therefore, within a same sample some pollen grains would be less competitive than others at the time of the pollination. Contrary to non-germinated pollen grains, those that germinated have a less dense intra-cellular content. This result would indicate that the pollen germination mobilizes some intra-cellular metabolites. It was reported that elongation of pollen tube during germination mobilizes some biochemical reactions (Colas & Mercier, 2000) [8].

In modeling approach of pollen fertility although positively correlation between pollen germination and fruit-set rates recorded in Dwarf coconut population this interrelationship was less strong than the one recorded in Tall coconut population. As reported in *Zea mays* L. (Cerović *et al.*, 2014) [26] the gene expression pattern of pollen tubes grown *in vitro* differs from that of pollen tubes grown through the stigma and style that would explain why Tall coconut was differed to the Dwarf. Our results were confirmed those from Regi *et al.* (2012) [27] studies on fruit-set in coconut upon artificial pollination, postulating that fruit-set is genotype dependent. Thus, beside pollen age and total sugars content, *in vitro* pollen germination was the best variable explaining more than 50% of fruit-set fluctuations in only Tall coconut population studied. *In vitro* pollen germination test appraises pollen capacity to germinate and to develop pollen tubes which is the mechanism alike the one that occurs in the natural conditions when pollen tubes grow on stigma then in pistil after pollination. Thus, *in vitro* pollen germination was the best indicator of fruit-set or seed production as mentioned by Colas & Mercier (2000) [8]. Hence, a total of 64% fruit-set rate fluctuations at the field was due to *in vitro* pollen germination from the most robust Eq. (2) developed in Tall coconut population studied. This model developed in controlled pollination process (Fig.1) and pollen storage conditions currently used by Marc Delorme Research Station (Côte d'Ivoire) could permit to foresee early, i.e. one year in advance, seed yields obtained by controlled pollinations. That must help considerably breeders and curators to correct the weak sample size of the progenies to consider in field trial designs, the representativeness of the parents palms used during field collection regeneration process by sexual reproduction and the lead time satisfactions of the improved seeds for farmers. In the order to efficient management of the pollen bank on Marc Delorme Research Station this model or Eq. (2) permits to deduct a critical level (29.14%) of stored pollen germination. Below this critical level of pollen germination rate, the pollen samples stored are not good quality because they give after hand pollinations null values of fruit-set rates. With the international norm of pollen quality, i.e. a level of pollen germination rate fixed to 35% (De Nuce *et al.*, 1980) [16], estimated fruit-set rate is 8.8% which gives a minimum average of 2 fruit-set per treated inflorescence. Model of fruit-set rate proposed in Tall coconut population in current study appears robust because the seed yield of controlled pollinations was comparable to the one reported by De Nuce *et al.* (1980) [16] using high pollen quality, i.e. a variation ranged from 3 to 4 seeds per pollinated bunch. Therefore, this mathematical model of fruit-set rate could be generalized. Nevertheless, 36% of fruit-set rate fluctuations obtained at the end from this mathematical model are not explainable by *in vitro* pollen germination rate. This gap could include depressive effects of pollination bag (Bhattacharya & Mandal, 2003) [28], climatic factors and technician blunder (Issali *et al.*, 2013) [15], pests and diseases (Doh *et al.*, 2014) [29] and natural abortion of fertilized female flowers per inflorescence on female palms to improve number of nuts coming to maturity. Herein, the results suggest that these abiotic and biotic effects with the exception of germinative capacity of pollen used that thwart the seed outputs at the field would be more important in Dwarf coconut populations than in the Tall.

Besides, stored pollen age and total sugars content were the best variables explaining 56.25 to 79.21% of the fluctuations of *in vitro* pollen germination rate through mathematical models that are Eq. (3) and Eq. (4) found in Tall and Dwarf coconut populations respectively. Likewise fruit-set, during storage coconut pollen germination is genotype dependent. Similar results were cited in some *Prunus persica* genotypes (Sharafi, 2011) [30] and *Prunus domestica* L. cultivars (Sharafi *et al.*, 2013) [31]. Total sugars content would influence pollen germinative capacity. Indeed, endogenous sugars of pollen represent its carbonated nutriments used in metabolism reactions during germination. Likewise, the links found between pollen age and biochemical contents of stored pollen such as total sugars and proteins contents were explained why the pollen age increase affected significantly *in vitro* pollen germination.

5. Conclusions

These investigations were permitted to show that fluctuations of coconut pollen fertility during cold storage at the laboratory could be explained by pollen age and some pollen biochemical components. Thus, *in vitro* pollen germination rate estimated at the laboratory was the best indicator having explained 64% of the fluctuations of the fruit-set rate assessed three month after pollination at the field. More than 50% variations of pollen germination rate were explained by pollen age and its total sugars content. Hence, in Côte d'Ivoire coconut breeders and curators could improve pollen bank management and plan better seed yields obtained by hand pollinations from these developed simple regression models thus far unknown.

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