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Research Paper

Manual pollination in kola (*Cola nitida*): A technique for increasing seeds production

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Abstract. The productivity of the kola tree is partly limited by a pollination deficit. Only 1.5 to 5% of flowers are pollinated and result in fruiting. To optimise nut production and meet the high demand for improved seed, a trial of manual pollination was carried out on eight genotypes. The results obtained with manual pollination were higher than those obtained with open pollination usually used for improved seed production. Indeed, the fruit set rate was 92.66% vs. 10.89%, the fruiting index was 0.42 vs. 0.06. Follicles and the number of nuts were increased to 800% in manual pollination. Furthermore, the mean of each parameter varied significantly between the eight genotypes, except fruit set rate ($p = 0.08$) and number of follicles harvested ($p = 0.11$). Finally, follicles and nuts numbers were significantly correlated with flowers number, $r = 0.63$ and $r = 0.62$ respectively, underhand pollination conditions. Finally, the results of this study strongly recommend the use of manual pollination for quantity production of improved kola seeds.

Keywords: *Cola nitida*, Côte d'Ivoire, fruiting index, manual pollination, open pollination, seeds.

INTRODUCTION

The Kola tree (Cola sp) is a tree native to Africa (Ghedira *et al*., 2009), and of economic interest to Côte d'Ivoire. An allogamous (Nyadanu *et al*., 2021) and diploid plant with 2n = 40 chromosomes (Adebola and Morakinyo, 2005), the kola tree is cultivated for its nuts, which are highly prized in many fields: modern and traditional medicine(Singh *et al*., 2012; Agbor *et al*., 2019; Ekalu and Habila, 2020), agri-food (Jayeola, 2001; Burdock *et al*., 2009; Sidibé *et al*., 2019) and textile industry for dye manufacturing (Asogwa *et al*., 2006). Kola nuts are also used in social relationships such as baptisms, weddings, funerals, etc. (Ouattara, 2013; Dah-Nouvlessounon *et al*., 2015).

Côte d'Ivoire is the world's largest producer and exporter of kola nuts since 2016, with an annual production of about 260,000 tonnes (FAO, 2019). This production is ensured not only by established kola plantations, but also by the exploitation of spontaneous trees or intercropped with other perennial crops such as cocoa, coffee and fruit trees (Deigna-Mockey *et al*., 2016; Ouattara, 2021).

Despite the importance and this performance of kola nut cultivation in Côte d'Ivoire, its production is facing significant difficulties, including the lack of quality plant materials and parasitic pressure. In Côte d'Ivoire, recent decades have been marked by increasing demands for quality plant material. Plant material used for kola nut cultivation comes either from cuttings, grafted plants or nuts (hybrids). However, difficulties have been observed, particularly with cuttings and grafts. For cuttings, these difficulties concern low success rates in the nursery and low recovery rates after transplanting (Séry *et al*., 2019, 2019, 2021; Traoré *et al*., 2020). For grafts, the most important constraint is the availability of both seeds for

Table 1. List of genitors used in kola tree pollination tests.

Genitors	Characteristics	Number of flowers used		
		Open pollination	Manual pollination	Total
A517	Precocious	107	131	238
A518	High productivity	88	90	178
A519	High productivity	136	111	247
A520	High productivity	87	107	194
A521	High productivity	104	101	205
AR6	High productivity	90	107	197
CIV-306	High productivity	82	99	181
CIV-313	High productivity	93	105	198
Total		**787**	**851**	**1 638**

rootstocks and scions' production, and grafting technicity (Traoré *et al*., 2019; Séry *et al.*, 2020). Thus, in the face of these constraints, the production and supply of plant material in quantity and quality in the form of seed is an alternative.

In kola trees, low tree productivity was noted despite the abundance of flowering and fruiting resulting from floral development. Reasons for this low productivity include incompatibility between genotypes and pollination deficiency (Morakinyo *et al*., 1981; Nyadanu *et al.*, 2021). In kola trees, pollination is carried out naturally by wind and insects. It results in the fruiting of only 1.5 to 5% of the formed flowers (Dublin, 1965). The same was observed on the cocoa tree (Lachenaud, 1991, 1995).

To overcome this constraint, different methods have been proposed: hormone-based treatments (Tarchoun and Dridi, 2005), mineral fertilization (Williams *et al*., 2003) and manual pollination (Lassen, 2016). Manual pollination has been successfully applied to cherimoya (*Annona cherimola*) by Ezzahouani *et al.* (1996), pear (*Pyrus communis*) by Flereau (2020) and African locus bean (*Parkia biglobosa*) by Lassen (2016). In the cocoa tree (*Theobroma cacao*), seed production has also been improved by manual pollination (Lachenaud, 1991, 1995; Bastide and Sounigo, 1993). In this study, the application of the manual pollination technique to *C. nitida* was presented, with the objective to optimize and increase seeds production.

MATERIALS AND METHODS

Plant materials

Eight genitors including two clones (CIV-306 and CIV-313) propagated by cuttings and six progenies (A517, A518, A519, A520, A521 and AR6) from seedlings were used in the trial. Clones CIV-306 and CIV-313 are known for their high productivity. The six progenies are also known for their high productivity and precocity, and are used as scions for the propagation of kola planting materials. These eight genotypes are all inter-compatible (Koné, 2019). A total of 787 flowers were observed for open pollination (OP) and 851 flowers for manual pollination (MP) (Table 1).

Implementation of open and manual pollinations

Pollinations were carried out in July, corresponding to the maximum flowering and natural fruit set. For open pollination (OP), the inflorescences were identified and tagged (Figure 1a) and the flowers were followed during their development. Each label had the date of identification and the number of flowers identified. At the level of manual pollination (MP), female flowers were first isolated. This isolation consisted of protecting them from anthesis the day before with a thin cloth sleeve (Figure 1b), in order to avoid any foreign pollen supply by insects or wind. Pollen was extracted on the day of pollination at anthesis (Nitsa *et al.*, 2020), gently brushing the anthers (Figure 1c). The extracted pollen was applied to the stigma of the female flowers in the morning between 07:00 and 11:00 am (optimum pollen germination period), lightly tapping the brush tuft with the pollen on the tip of the stigma (Figure 1d). After pollen application, each inflorescence was tagged with the date of pollination and the number of flowers pollinated. The flowers were again protected from foreign pollen with a sleeve. The sleeves were removed 48 h after pollination.

Data collection

The data collected included the number of knotted flowers (NFN), and the number of follicles (NFOL) and the number of harvested nuts (NoGr). From these variables, the fruit set rate (TN), follicle retention rate (TM), fruiting index (IF), number of follicles and number of harvested nuts were estimated. Fruit set rate (TN) was determined seven days after pollination, using equation 1:

$$TN = \frac{NFN}{NFT} \times 100 \qquad (1)$$

Figure 1. Steps of manual pollination in kola tree. a) Inflorescences identified in open pollination, b) isolated inflorescences with a sleeve, c) pollen collection with a brush, d) pollination of a female flower.

Table 2. Means of parameters determined in open and manual pollination among eight kola genitors.

Variables	Open pollination	Manual pollination	df	*t* test value	p
TN (%)	10.89 ± 5.02^{B}	92.66 ± 4.95^{A}	46	-56.79	< 0.001
TM (%)	57.44 ± 18.58^{A}	45.67 ± 7.46^{B}	46	2.88	0.006
IF	0.06 ± 0.03^{B}	0.42 ± 0.07^{A}	46	-20.93	< 0.001
NFOL	9.87 ± 5.3^{B}	74.79 ± 16.73^{A}	46	-18.11	< 0.001
NoGr	61.79 ± 37.04^{B}	488.17 ± 141.83^{A}	46	-14.24	< 0.001

Mean with the same letters on the same line are statistically equal at the 5% level.
TN: fruit set rate; TM: follicle retention rate; IF: fruiting index; NFOL: number of follicles harvested; NoGr: number of nuts harvested.

TN = fruit set rate; NFN = number of knotted flowers; NFT = number of total pollinated or observed flowers

Follicle retention rate (TM) and fruiting index (IF) were, assessed 80 days after pollination, using Equations 2 and 3, respectively.

$$TM = \frac{NFOL}{5 \times NFN} \times 100 \quad (2) \qquad IF = \frac{NFOL}{5 \times NFT} \quad (3)$$

TM = Follicles retention rate; NFOL = Number of follicles formed; IF = fruiting index

Statistical analysis

The means of all the variables evaluated for the two types of pollination (treatment) were compared by the Student t-test at the 5% level. The eight genitors were compared by variance analysis to a classification criterion (the genotype) at the 5% level for each treatment. Within each genotype, the treatments were compared by the same method with the SAS 9.4 software. The quantities of follicles and nuts harvested in manual and open pollination were represented on a histogram on an Excel table. The relationships between the number of pollinated (or observed) flowers and the number of harvested nuts were determined by linear regression using the Statistica 7.1 software.

RESULTS

The results presented in Table 2 show a significant difference between open pollination (OP) and manual pollination (MP) for all variables. The fruit set rate of the flowers obtained in manual pollination (92.66%) was significantly higher than that obtained in open pollination (10.89%). The same was true for the fruiting index (0.42 in MP vs 0.06 in OP; $t = -20.93$; $p < 0.001$). In relation to the follicles and number of nuts, an average of 74.79 follicles and 488.17 nuts were obtained in manual pollination, compared with 9.87 follicles and 61.79 nuts in open pollination ($p < 0.001$). Which represents an increase of about 800% in follicles and nuts. In contrast, the retention rate of follicles obtained in open pollination was higher than the value obtained in manual pollination (57.44% in OP

Table 3. Fruit set rates obtained in open and manual pollinated flowers in eight kola genitors.

Genotypes	Open pollination (%)	Manual pollination (%)	F	p
CIV-306	8.46 ± 1.36 $^{B\ b}$	94.41 ± 2.13 $^{A\ a}$	3446.76	< 0.001
CIV-313	16.26 ± 3.95 $^{B\ a}$	97.11 ± 0.33 $^{A\ a}$	1244.53	< 0.001
A517	8.27 ± 1.89 $^{B\ b}$	92.29 ± 4.83 $^{A\ a}$	785.74	< 0.001
A518	9.04 ± 3.72 $^{B\ b}$	87.24 ± 8.68 $^{A\ a}$	205.45	< 0.001
A519	16.43 ± 3.22 $^{B\ a}$	90.12 ± 2.86 $^{A\ a}$	875.58	< 0.001
A520	8.02 ± 1.75 $^{B\ b}$	89.09 ± 3.88 $^{A\ a}$	1087.18	< 0.001
A521	4.85 ± 1.81 $^{B\ b}$	93.97 ± 2.85 $^{A\ a}$	2086.63	< 0.001
AR6	15.77 ± 4.7 $^{B\ a}$	97.05 ± 2.86 $^{A\ a}$	653.71	< 0.001
Mean (%)	10.89	92.66		
F	6.71	2.22		
p	< 0.001	0.08		

Mean with the same capitals letters in the same line are statistically equal at the 5% level.
Mean with the same lower-case letters in the same column are statistically equal at the 5% level.

Table 4. Variation in follicle retention rate among the eight kola genitors under open and manual pollination.

Genitors	Open pollination (%)	Manual pollination (%)	F	p
CIV-306	51.11 ± 10.18 $^{A\ ab}$	47.91 ± 0.44 $^{A\ ab}$	0.30	0.61
CIV-313	74.44 ± 5.09 $^{A\ ab}$	44.37 ± 3.72 $^{B\ ab}$	68.17	0.001
A517	52.77 ± 19.31 $^{A\ ab}$	47.01 ± 3.7 $^{A\ ab}$	0.26	0.63
A518	66.67 ± 15.27 $^{A\ ab}$	53.06 ± 4.39 $^{A\ a}$	2.20	0.21
A519	37.02 ± 14.46 $^{A\ b}$	33.61 ± 6.94 $^{A\ c}$	0.14	0.73
A520	37.78 ± 10.71 $^{A\ b}$	39.91 ± 4.82 $^{A\ bc}$	0.10	0.76
A521	76.67 ± 15.27 $^{A\ a}$	46.1 ± 6.59 $^{B\ ab}$	10,12	0.03
AR6	63.11 ± 14.68 $^{A\ ab}$	53.43 ± 5.12 $^{A\ a}$	1,16	0.34
Means (%)	57.44	45.67		
F	3.73	5.5		
p	0.014	0.002		

Mean with the same capitals letters in the same line are statistically equal at the 5% level.
Mean with the same lower-case letters in the same column are statistically equal at the 5% level.

compared to 45.67% in MP; t = 2.88; p < 0.001).

Fruit set rate

The fruit set rate showed a significant difference between the eight open-pollinated genotypes (p < 0.001). Average values for fruit set rate ranged from 4.85% to 16.43% (Table 3). Genitors CIV-313, A519 and AR6 had the highest averages for fruit set rate. On the other hand, fruit set values obtained in manual pollination showed no significant differences between the eight kola genitors (p = 0.08).

Follicles retention rate

Follicles retention rates varied significantly among the eight kola genitors in both open pollination (p = 0.014) and manual pollination (p = 0.002). Averages ranged from 37.02 to 76.67% in open pollination and from 33.61 to 53.43% in manual pollination (Table 4). Genitor A521 had the highest retention in open pollination, while the mean was significantly lower in genitor A519 (37.02%) and A520 (37.78%). In manual pollination, the highest follicles retention rate was observed in genitor A518 (53.06%) and AR6 (53.43%); while the lowest was observed in genitor A519 (33.61%) (Table 4).

Fruiting index

Fruiting index was significantly different among the eight kola genitors in each treatment (open pollination: p < 0.001; manual pollination: p = 0.002). Means ranged from 0.03 to 0.12 in open pollination, and from 0.30 to 0.52 in manual pollination (Table 5). Genitors CIV-313 and AR6 had the highest averages for the fruiting index under open pollination. These means were of the same order of magnitude (0.12 and 0.11 respectively). In contrast,

Table 5. Variation in fruiting index among the eight kola genitors under open and manual pollination.

Genitors	Open pollination	Manual pollination	F	p
CIV-306	0.04 ± 0.01 $^{A\ b}$	0.45 ± 0.01 $^{B\ ab}$	1568.64	< 0.001
CIV-313	0.12 ± 0.03 $^{B\ a}$	0.43 ± 0.03 $^{A\ ab}$	126.46	< 0.001
A517	0.04 ± 0.01 $^{B\ b}$	0.43 ± 0.03 $^{A\ ab}$	478.61	< 0.001
A518	0.06 ± 0.01 $^{B\ b}$	0.46 ± 0.08 $^{A\ ab}$	75.32	0.001
A519	0.05 ± 0.01 $^{B\ b}$	0.30 ± 0.06 $^{A\ c}$	45.22	0.002
A520	0.03 ± 0.01 $^{B\ b}$	0.36 ± 0.06 $^{A\ bc}$	90.84	< 0.001
A521	0.06 ± 0.01 $^{B\ b}$	0.43 ± 0.05 $^{A\ ab}$	151.47	< 0.001
AR6	0.11 ± 0.02 $^{B\ a}$	0.52 ± 0.05 $^{A\ a}$	165.00	< 0.001
Means	0.06 ± 0.03	0.42 ± 0.07		
F	8.76	5.24		
p	< 0.001	0.002		

Mean with the same capitals letters in the same line are statistically equal at the 5% level.
Mean with the same lower-case letters in the same column are statistically equal at the 5% level.

Table 6. Variation in the number of follicles among the eight kola genitors under open and manual pollination.

Genitors	Open pollination	Manual pollination	F	p
CIV-306	6.00 ± 2.00 $^{B\ cd}$	79.00 ± 14.42 $^{A\ a}$	75.41	0.001
CIV-313	18.67 ± 4.16 $^{B\ a}$	75.00 ± 4.35 $^{A\ a}$	262.03	< 0.001
A517	7.67 ± 3.05 $^{B\ bcd}$	94.67 ± 8.38 $^{A\ a}$	285.03	< 0.001
A518	8.33 ± 1.53 $^{B\ bcd}$	70.67 ± 20.03 $^{A\ a}$	28.88	0.005
A519	13.33 ± 4.51 $^{B\ abc}$	55.67 ± 9.29 $^{A\ a}$	50.40	0.002
A520	4.33 ± 1.15 $^{B\ d}$	65.33 ± 26.76 $^{A\ a}$	15.55	0.01
A521	6.67 ± 3.21 $^{B\ bcd}$	72.66 ± 13.61 $^{A\ a}$	66.79	0.001
AR6	14.00 ± 2.00 $^{B\ ab}$	85.33 ± 5.85 $^{A\ a}$	398.23	< 0.001
Means	9.87	74.79		
F	8.42	1.99		
p	< 0.001	0.11		

Mean with the same capitals letters in the same line are statistically equal at the 5% level.
Mean with the same lower-case letters in the same column are statistically equal at the 5% level.

genitors CIV-306, A517, A518, A519, A520 and A521 had the lowest fruiting index. The means were also similar.

Number of follicles harvested

The number of follicles harvested varied significantly from one genitor to another ($p < 0.001$). Genitors produced between 4.33 and 18.67 follicles from open pollination. Clone CIV-313 produced the most follicles, while genotype A520 produced significantly fewer follicles (Table 6). In contrast, the number of harvested follicles increased considerably in manual pollination. The mean number of follicles ranged from 55.67 for A519 to 94.67 for A517 but did not vary significantly between genitors ($p = 0.11$).

Number of nuts harvested

The kola genitors produced between 21.67 and 130.67 open-pollinated nuts, with an average of 61.79 (Table 7). This number increased considerably in manual pollination (320.67 to 692 nuts) with an average of 488.17 nuts. This corresponds to a gain of more than 600%. In each treatment, the means were significantly different from one genitor to another (OP: $p < 0.001$; MP: $p = 0.002$). The number of nuts produced was significantly higher in the CIV-313 clone in each treatment, as was the A521 genitor with the lowest number of nuts.

Relationship between the number of observed or pollinated flowers, and the number of harvested nuts

The correlation estimated between the number of flowers observed and the number of harvested nuts is not significant ($r = 0.08$; $p = 0.71$). This relationship indicates that the number of harvested nuts does not increase with the number of flowers observed (Figure 2a). However, in manual pollination, there is a strong correlation between the number of pollinated flowers and the number of harvested nuts. This correlation is positive and significant

Table 7. Variation in the number of nuts harvested among the eight kola genitors under open and manual pollination.

Genitors	Open pollination	Manual pollination	F	p
CIV-306	36.00 ± 12.00 [B c]	559.00 ± 124.89 [A ab]	201.13	0.002
CIV-313	130.67 ± 29.14 [B a]	692.00 ± 93.00 [A a]	99.52	< 0.001
A517	38.33 ± 15.27 [B c]	570.67 ± 53.87 [A ab]	271.12	< 0.001
A518	50.00 ± 15.00 [B c]	337.33 ± 94.58 [A bc]	27.01	0.006
A519	66.66 ± 22.55 [B bc]	454.33 ± 25.42 [A bc]	390.46	< 0.001
A520	21.67 ± 5.77 [B c]	432.67 ± 152.72 [A bc]	21.65	0.009
A521	55.00 ± 15.00 [B c]	320.67 ± 65.85 [A c]	46.42	0.002
AR6	96.00 ± 12.00 [B b]	539.00 ± 20.00 [A abc]	1082.26	< 0.001
Means	61.79	488.17		
F	12.99	5.87		
p	< 0.001	0.002		

Mean with the same capitals letters in the same column are statistically equal at the 5% level.
Mean with the same lower-case letters in the same line are statistically equal at the 5% level.

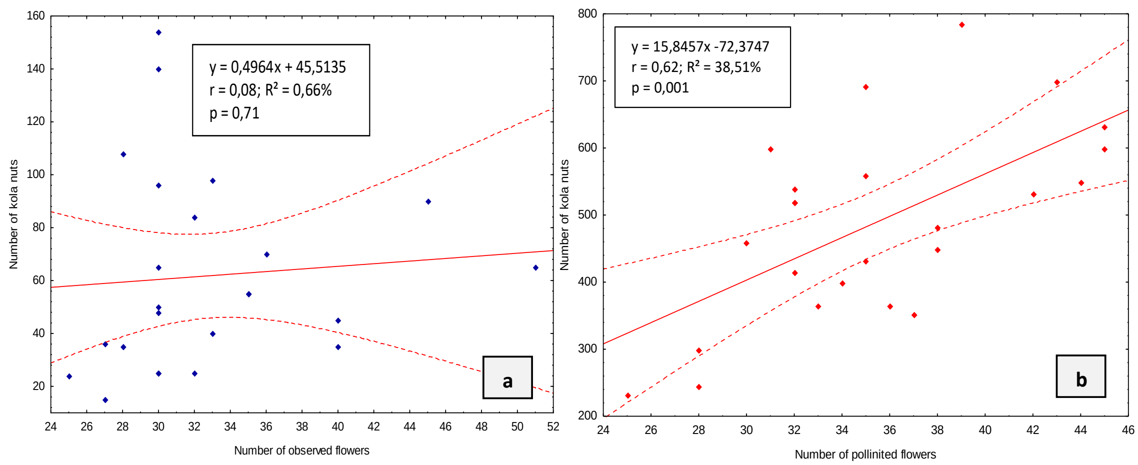

Figure 2. Relationship between the number of follicles harvested and number of flowers observed under open pollination (a) and manually pollinated flowers (b) among eight kola genitors.

(r = 0.62; p = 0.001). Thus, in the genitors involved, harvested nuts increase with the number of pollinated flowers (Figure 2b).

The combined production of follicles and nuts confirms the previous results. Thus, out of a total of 787 flowers observed in open pollination for the eight genitors, 237 follicles were collected (Figure 3). Regarding nut number, 1,483 nuts were harvested, i.e. 7% of success rate. However, in manual pollination, the yield was significantly improved: 1,785 follicles were harvested for 849 pollinated flowers. This represents a rate of 42%, and 11,716 nuts, i.e. 55% of success rate.

DISCUSSION

The results of this study showed significant differences between the eight genitors for all variables measured, with the exception of fruit sets that did not differ significantly in manual pollination. These variations may be due to the differences in the genetic composition of the individual kola genitors and the effects of the environments in which the genitors were established (Lawin *et al.*, 2021). Indeed, each tree has its own micro-environment. In addition, six genotypes (A517, A518, A519, A520, A521 and AR6) involved in the study are derived from seeds and clones (CIV-306 and CIV-313) which were grafted are genetically different (Ouattara, 2021). Given the allogamous nature of *C. nitida* (Nyadanu *et al.*, 2020), genotypic variations observed in this study for fruit set rate, follicle retention rate, fruiting index, number of follicles and number of nuts could be due to genetic effects. These results are consistent with those of Beli *et al*. (2009) who reported variations in fruiting on different rubber clones. According to these authors, these variations could be dependent on genetic factors. Similarly, Makueti *et al.* (2012) demonstrated the influence of the parent and pollen type on fruiting in Dacryodes edulis in Cameroon.

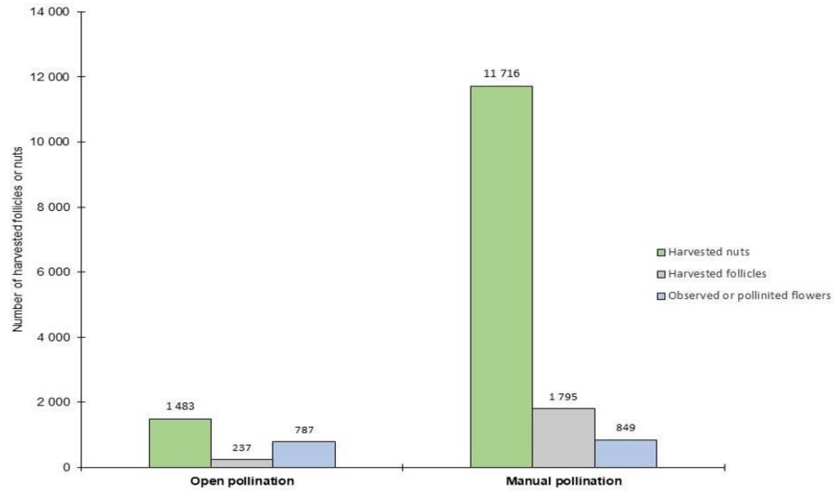

Figure 3. Histogram of cumulation of follicle and nut production in open pollination and manual pollination in eight kola genitors.

Fruit set rates were very high in manual pollination and very low in open pollination. This may be due to a lack of effective pollinators in natural conditions, as indicated by Pastou *et al*. (1999) in *Annona squamosa* where open pollination results in very low fruit set rates (1.5 to 2% of flowers). Other hypotheses could justify this finding. First, the amount of pollen that gets into contact with the stigma may be larger after manual pollination than under open pollination conditions. Because the pollen is collected directly from the male flower, the operator ensures that the pollen grains are actually extracted in sufficient quantity. This can be seen by the presence of whitish powder on the brush tuft.

There is also the period of manual pollination, which took place in the morning as part of our study. Indeed, Bakayoko *et al*. (2019) demonstrated that the best time of practice for manual pollination is in the morning between 7:00 am and 11:00 am, after which the results of pollination obtained are less efficient. Thus, it is not always clear that pollinating insects visit flowers during this period. This can help reduce fruit set rates in open pollination.

Pollen quality and viability could also be affected (Nyadanu *et al*., 2023). In fact, manual pollination is carried out with pollen collected just after anthesis. However, free pollination is done by insects. These insects can collect pollen several hours after anthesis and carry it for long periods before landing on the stigma. As pollen viability does not exceed forty-eight hours under natural conditions (Nitsa *et al*., 2020), it may be thought that the pollen brought manually is more viable and fertile than that carried by insects.

Stigma responsiveness also plays an important role in fruit set (González *et al*., 1995; Mesejo *et al*., 2007). In the kola tree, the duration of stigma receptivity is four days (Dublin, 1965) after which it cannot germinate pollen and develop pollen tubes to ensure fertilization (Sanzol *et al*., 2003; Brevis *et al*., 2006). Despite this relatively long period, low fruit set rates were observed in open pollination in this study. This presupposes that in a kola tree plantation, there may be no supply of pollen during the period of receptivity of the stigma. This causes flowers to drop and low fruit set rates as mentioned by Bastide and Sounigo (1993).

Self-incompatibilities would also contribute to low fruit set rates and low production observed in open pollination (Morakinyo *et al.*, 1981; Odutayo *et al*., 2018). Indeed, some self-pollination may seem inevitable when the dehiscence of some anthers and the receptivity of the stigma overlap inside the flowers. This has already been highlighted by Vaughton *et al.* (2010) in *Cyrtanthus breviflorus*. These overlaps promote intra-seasonal self-pollination by pollinators. Since the kola tree is allogamous, it is obvious that self-pollination and the incompatibility of this self-pollen cause degeneration of female gametes not fused with male gametes. Which could limit the development of the ovaries. Moreover, in addition to the phenomenon of self-incompatibility, it is possible to admit that self-pollen is less fertile than pollen coming from another male parent. Indeed, an effect of pollen genotype on fruiting was demonstrated by Lachenaud (1991, 1995) in cocoa trees.

Finally, the pollinating insect population is a determining factor in pollination (Cilas *et al*., 1984; Meynié and Bernard, 1997). Indeed, in the oil palm, Mariau *et al.* (1991) suggest that the rate of flower fruit set would depend in part on the composition of pollinating insect populations. The authors reveal that *Elaedobius subvittatus* is a lesser pollinator than *E. kamerunicus*. By establishing the relationship between fruit set rates and percentages of *E. subrittatus* in a given population, the researchers have shown that the higher the populations are rich in *E. subvittatus* (hence poor in *E. kamerunicus*), the lower the fruit set rates. In the kola tree, research on pollinators has shown that the most common are *Oecophylla maraydina* and *Torma colae* (Dublin, 1965). *Torma colae* is a constant-winged insect. But the evidence that it represents a pollinating agent has not been elucidated because none of the specimens observed had pollen on the body. This “deficit” of pollinating insects would be another factor explaining the low fruit set rates of flowers in open pollination. However, quantitative and specific analysis of insect populations visiting flowers would provide more precision.

Manual pollination resulted in higher fruit set rates than under natural conditions. But the trend is reversed for follicle retention rate. In addition, genotypes that had the highest fruit set rates had the lowest follicle retention rates. This result shows that when the fruit set rate is high, trees lose more follicles than those with a low fruit set rate. This result is consistent with those of Makueti *et al*. (2012) in *Dacryodes edulis* and could be explained by the natural self-regulation of tree fruiting. One could also invoke a physiological shedding (Oladokun, 1988) related to a congestion of the formed follicles. This congestion can lead to competition on the one hand between the developing follicles, and on the other hand between the follicles and growing vegetative organs, for mineral substances and growth hormones. In addition, the phenomenon of follicle drop is probably also controlled by growth hormones such as ethylene (Hilt and Bessis, 2003). Ethylene is one of the phytohormones associated with fruit maturation. It causes cell turgescence, and in this way, it also causes cell wall destruction. This results in the activation of the abscission zone of the follicle causing its drop. On the other hand, the drop in the follicles could be due to parasitic causes or attacks by pests (Bos *et al*., 2007; Amonmidé *et al*., 2020).

The number of follicles and nuts produced as a result of open pollination was much lower than that produced by manual pollination. This confirms once again that manual pollination significantly improves tree production. Similar results were obtained on cocoa trees (Bastide and Sounigo, 1993) where the average number of beans per pod was higher after manual pollination than after open pollination. In addition, our study found a correlation between the number of pollinated flowers and nuts number in manual pollination. This was not the case in open pollination where the correlation was low and not significant. This low correlation supports the hypothesis that flowering abundance does not necessarily mean significant fruiting (Lachenaud, 1991). This is due to a significant drop in flowers under natural conditions in the kola tree even before the fruit set (Oladokun, 1988). It would therefore be wise to pollinate a large number of flowers in order to have good production. However, it is necessary to limit the number of flowers to be pollinated by inflorescence, which means from one to four flowers per inflorescence (Badiba, 2018). Indeed, a high number of flowers knotted by inflorescence favour the congestion of the follicles, and consequently their drop.

Conclusion

Manual pollination improved the total production of the eight kola tree genotypes involved in the study. This improvement has been reflected on the one hand by the induction of high fruit set rates, and on the other hand by the number of follicles and nuts harvested which have greatly increased. Manual pollination would be more beneficial and would be a strategy for improved yields and profitability of the kola tree. In addition, to meet the growing demand for seeds, the application of the manual pollination technique to the kola tree could be an alternative to increase seed production.

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