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Compatibility of Ten Elite Cocoa (*Theobroma cacao* L.) Clones

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Abstract: One way of boosting cocoa productivity which has plummeted over the last decade in Côte d'Ivoire is to introduce high performing clones. Preliminary observations have indicated that these new clones have differing growth patterns and agronomic traits in the field. Assessing their compatibility is of paramount importance since these clones will be made available to the farmers in the near future. This study was conducted in Soubré, southwest of Côte d'Ivoire, to evaluate the compatibility of ten new cocoa clones (coded as C1, C8, C9, C14, C15, C16, C17, C18, C20, and C21). A half diallel design consisting of 10 self-pollinations and 45 inter-crosses, replicated three times, was used. Results showed significant differences among clones for pollination success. Out of the ten clones evaluated, six (C1, C8, C9, C15, C17 and C21) were self-compatible. With a 39% mean pollination success, C9 was quite interesting because it has been reported to be among the highest yielding clones. Introduction of improved germplasm will go a long way towards enhancing productivity in Côte d'Ivoire, radically impact farmers' livelihoods, and contribute significantly to a more reliable supply of cocoa beans for chocolate manufacturers.

Keywords: self-pollination; inter-pollination; pollination success; Côte d'Ivoire

1. Introduction

Cocoa (*Theobroma cacao* L.) is a preferentially allogamous tropical woody species native to South America, belonging to the Malvaceae family [1]. *T. cacao* is a heterozygous plant with a high variability for agronomic and quality traits [2,3]. Several studies have shown that the flowers of cocoa are androgynous and essentially fertilized by midges (*Forcipomyia* spp.) [4–6]. Each cocoa tree can produce up to 125,000 flowers per year [6,7], each flower producing up to 14,000 pollen grains [8] and up to 74 ovules [9]. Anthesis and anther dehiscence occur during the dawn hours and the flowers are accessible for one day; the stigma and style are most receptive in the morning and early afternoon [10]. However, at the end of the receptivity period, 50% to 75% of the flowers are unpollinated and drop from the tree [11,12]. Finally, only 2% of the flowers produce mature fruit [13–15] due to fruit abortion [16–18]. More recently, it has been reported that the number of pollinated and fertilized flowers that progress to fruit is, on average, three out of 1000 flowers [19].

Sexual reproduction in numerous flowering plants includes self-incompatibility, which is one of the most important mechanisms to avoid inbreeding [20]. Although most cocoa plants are self-incompatible, and are hence reliant on cross-pollination by midges, recent studies have shown evidence of self-pollination in wild and cultivated cocoa [21]. Inter-pollination success in cocoa ranges from 18% to 66% [22], while this rate is up to 100% with self-incompatible clones [23]. In 1932, Pound first reported self-incompatibility in *T. cacao* [24]. Appraisals on the mechanism of self-incompatibility

in cocoa indicated that incompatible pollination did not inhibit pollen germination or pollen tube evolution [25]; compatible and incompatible pollination resulted in similar pollen tube growth rates [25,26]. As such, Theobroma symbolizes a classic illustration of an ovarian auto-incompatibility method, to a certain extent different from most plant incompatibility methods in nature [18,27].

Studies on self-compatibility have significance for obtaining and maintaining the genetic purity of cocoa clones and also for improvement and utilization of cocoa. Research conducted in Côte d'Ivoire has led to the development of some high performing clones whose compatibility is not known and needs to be documented as part of an overall genetic characterization program prior to introducing them to farmers. This could boost farms' yields and improve the farmer's livelihood in the main cocoa growing region of Soubré in Côte d'Ivoire. Recent findings have indicated that cocoa yield limitation seems to be determined by pollination rather than by resources [28]. Only if a cocoa plantation is more diverse and not a monoculture and when plant species share pollinators can the potential for transfer of hetero-specific pollen be high. However, the receipt of hetero-specific pollen can reduce seed production [29]. Ten clones were evaluated during this study with two control: clones C8 and C21. C8 is known as self-incompatible and C21 as self-compatible, but no information exists for the rest of the clones.

Previous studies on this subject showed that several observation dates for assessing pollination success (5, 10, 15, and 25 days after pollination) were used, concluding that 10 days after pollination was the time when there was optimum pollination success [30–32]. Indeed, 25% pollination success was suitable to designate a clone as self- or inter-compatible [30,32], while 20% pollination success was also used [31]. In this study, we attempted to identify the compatibility of cocoa clones with a level of $\geq$30% at 10 days after hand pollination. A clone which is self- or inter-compatible at this threshold (30%) will certainly yield more and be more stable compared to one with a lower threshold. However, it is known that the potential productivity of a clone is dependent on its levels of pollination success for both self- and inter-compatibility. The goal of this work was therefore to assess the self-compatibility and inter-compatibility of ten elite cocoa clones available in Côte d'Ivoire.

2. Materials and Methods

2.1. Site

The trial used 25-year-old cocoa trees grafted with the ten clones at a farm in Koda, located 12 km from Soubré, in the Nawa Region (southwest of Côte d'Ivoire, 5°47′08″ N, 6°36′30″ W, and 276 m a.s.l.). The mean annual rainfall is 1320 mm and varies from 1443 to 1199 mm. The temperature varied from 25 to 30 °C.

2.2. Plant Material

Scions obtained from ten (10) cocoa clones growing at the Centre National de Recherche Agronomique (CNRA) Station in Divo and coded as C1, C8, C9, C14, C15, C16, C17, C18, C20, and C21 were side grafted onto mature cocoa trees. The origin of these clones and potential yields are presented in Table 1.

Table 1. Origin and potential yield of the ten cocoa clones used in this study.

Clones	Origin	Potential Yield (ton/ha) (2011–2015) *
C1	Trinidad	2.3
C8	Trinidad	1.8
C9	Trinidad	2.3
C14	Côte d'Ivoire	2.8
C15	Côte d'Ivoire	2.0
C16	Côte d'Ivoire	4.0
C17	Côte d'Ivoire	1.9
C18	Côte d'Ivoire	1.4
C20	Côte d'Ivoire	1.4
C21	Côte d'Ivoire	1.6

* Data from the Vision for Change Project.

2.3. Experimental Design and Cultural Practices

The experimental design was a half diallel consisting of ten self-pollinations and forty-five inter-pollinations (Table 2) replicated three times. A total of thirty flowers per clone per replication were pollinated manually to determine the self- and inter-compatibility of the ten clones. Hand pollination was carried out from April to October 2015 using standard protocol [33].

Table 2. Half-diallel design of self- and inter-pollination crosses of the ten clones.

Clones	C1	C8	C9	C14	C15	C16	C17	C18	C20	C21
C1	X *	X	X	X	X	X	X	X	X	X
C8		X	X	X	X	X	X	X	X	X
C9			X	X	X	X	X	X	X	X
C14				X	X	X	X	X	X	X
C15					X	X	X	X	X	X
C16						X	X	X	X	X
C17							X	X	X	X
C18								X	X	X
C20									X	X
C21										X

* X = cross.

Flowers to be pollinated were identified the day before at 4.00 p.m. The criteria used were the flower size and the beginning of the separation of sepals (Figure 1a). The flowers were isolated with a piece of PVC tube (no more than two flowers per tube). The PVC tube was about 7 cm long and 2 to 2.5 cm in diameter (Figure 1b). The distal end was covered with a gauze fastened with an elastic band, and the base was fixed to the tree with a ring of modeling clay. The following morning (at about 6.30 a.m.), the tube was removed. The recently opened flowers were emasculated with a tip pincer by eliminating the staminodes to uncover the stigma. The pollen grains were manually removed from the flower and dropped on the stigma of an isolated flower on a same or a different tree for fertilization to avoid self-incompatibility and self-pollination. After pollination, the tube was quickly closed to prevent the entry of any pollinator. Care was taken to clean the tools and hands with diluted alcohol (70%) after every pollen transfer. The tube was removed three days after pollination. Pod formation of crosses is shown in Figure 1c.

(**a**) Hand pollination

(**b**) Isolation of pollinated flower

(**c**) Pod formation of C1 X C9 cross

Figure 1. Flower pollination process (**a**) Hand pollination; (**b**) Isolation of pollinated flower (**c**) Pod formation of C1 X C9 cross.

2.4. Data Collection

Data were collected 10 days after pollination (DAP). Pollination success was assessed as the percentage of the total number of pollinated flowers. Clones were rated as self-compatible or inter-compatible when the percentage of successful pollinations was ≥30%. The inter-compatibility was measured through the General Combining Ability of each clone (GCA). The GCA of a clone is the means of pollination success following the crossing of a given clone with the nine other clones.

2.5. Data Analysis

The pollination success was subjected to one-way analysis of variance (ANOVA) with the general linear model of Statistical Analysis System software [34]. Mean separation for each parameter was determined with the Duncan test using $p < 0.05$.

3. Results

3.1. Pollination Success

Analysis of variance revealed a significant difference among clones for pollination success for self-pollinations and for the inter-clone crosses (Table 3). It was observed that the mean pollination success for self-pollination and inter-pollination of cocoa clones was 17% and 26% for inter-pollination and self-pollination, respectively.

Table 3. Self- and inter-pollination success (%) of clones 10 days after pollination (DAP).

	Pollination Success (%)		
Clones	**Self-Pollination**	**Inter-Pollination**	**Mean**
C1	33.33 ab *	22.22 ab	27.78
C8	33.33 ab	16.30 abcd	24.82
C9	53.33 a	24.07 a	38.70
C14	16.67 bc	21.48 ab	19.08
C15	33.33 ab	14.82 bcd	24.98
C16	3.33 c	17.78 abcd	10.56
C17	30.00 ab	19.63 abc	24.82
C18	3.33 c	14.44 bcd	8.89
C20	13.33 bc	9.63 d	11.48
C21	36.67 ab	12.22 cd	24.45
Means	25.67	17.26	21.47
P	0.0024	0.0015	
CV	50.80	79.18	

* Values followed by different letters in each column are significantly different where $p \leq 0.05$ (Duncan).

3.2. Compatibility of Cocoa Clones

3.2.1. Self-Compatible Clones

Results showed a significant effect, indicating that the pollination success varied among clones. All ten clones exhibited pods after self-pollination but to varying degrees. The pollination success ranged from 3% for clones C16 and C18 to 53% for clone C9 (Table 3). One of the two control clones, C8 (reported as self-incompatible) appeared to be self-compatible with a pollination success of 33%; the other control (C21), self-compatible, had a pollination success of 37%. The results of the study showed that clones C1, C8, C9, C15, C17 and C21 could be designated as self-compatible.

3.2.2. Inter-Compatible Clones

Inter-compatible pollination success varied among clones (Table 3). Pollination success ranged from 9% for clone C20 to 24% for clone C9, followed by clones C1 (22%) and C14 (21%). Ten clones also bore low pods after inter-pollination.

4. Discussion

Six of the clones (C1, C8, C9, C15, C17 and C21) appeared to be self-compatible. Clone C9 showed a high level (53%) of self-pollination. These results are greater than those of [35] who obtained a pollination percentage of 43% in self-compatible cocoa trees. However, among these clones, one of the two controls, clone C8, showed successful self-compatibility despite the fact that it had been identified as self-incompatible. This may be due to environmental effects. Indeed, the environment may have played a role in the expression of some compatibility genes or traits [3,36]. The different levels of compatibility observed were a visible phenomenon indicated by the dropping of flowers, which could also be due to physiological traits and or environment conditions such as wind, rainfall, etc. This dropping was certainly correlated to floral abscission [14]. Flower abscission in cocoa happens within two to three days after anthesis if fertilization does not occur [37]. Unpollinated flowers drop after approximately two days, and pollinated flowers may also fall without setting due to scant pollen grains on the stigma [38]. Moreover, the significance of pollination can be governed by the degree of pollen compatibility, the number of pollen particles on the stigma and the potential productivity of the clones [39].

Inter-pollination success in cocoa has been reported to range from 18% to 66% [22]. According to our study, inter-pollination success was too low for an acceptable yield. The results obtained were much lower than self-pollination. These results concur with those of other authors who studied cocoa clones different from the ones evaluated in the current study [38] and on Prunus species [39]. A possible explanation for this phenomenon could be that in self-pollinated clones, the stigma and the stamens are in the same flower, making this very convenient for pollination to take place.

Concerning both self-compatibility and inter-compatibility based on our threshold, no clone appeared to have these characteristics despite the presence of some pods. This is interesting because other clones have shown contrary results [32]. These differences could be due to genotype but warrant further study.

The results of the means of self-pollination and inter-pollination success showed that C9 had the highest mean value (38.70%) compared to the other clones. This clone was among those which showed good yields in several previous studies in the Nawa region (ICRAF unpublished data). However, the clone C16 had higher yield potential than C9. A possible explanation could be due to the fact that compatibility and potential yields are not necessarily related [32].

5. Conclusions

This study showed different levels of compatibility of ten elite cocoa clones, indicating possible differences in their productivity. Clone C16 and to some extent clone C14 had good yield potential and could play a big role in improving small-holder farm productivity. On the basis of the means of self-pollination and cross-pollination success, clone C9 combined with clone C1 could be made available to farmers to enhance pollination success and consequently improve yields.

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Conflicts of Interest: The authors have no conflict of interest.

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