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Production of Hybrid Seeds by Intraspecific Crossing in Yam *Dioscorea alata* L

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Abstract: ***Manual crosses were carried out over two successive years on Dioscorea alata for the production of hybrid seeds between five females and seven males' parents with contrasting characteristics. A total of 22951 flowers were manually pollinated in both years (14145 in 2016 and 8806 in 2017) in 33 parental combinations. The ploidy levels of the parents were determined by flow cytometry. The crossed parents were all diploids except the female OA49 which was triploid. In both years the fruiting and seed rates were comparable (25.06% and 22.82%; 30.05% and 30.18%). A significant variation in fruiting rates was observed between the different parental combinations. Analysis of variance have shown that there is both a male effect on the fruit setting. The lowest rates were observed in combinations involving male clones 23 and Ma01 (2.04% and 2.77%) and the highest rates involved males TDa00/00128 and TDa00/00095 (48.25% and 33.91%). The triploid female OA49 is sterile. Females TDa01/00003, TDa01/00018, TDa99/00240 and TDa01/00295 gave comparable fruiting rates which are respectively (37.87%, 29.38%, 24.70% and 20.92%). For seeds production, there is no male and female effect. The fruiting rate according to the time slot depends on each male variety involved. For some males, the fruiting rate is influenced by the time slot of pollination while for others, there is no time slot effect on the fruiting rate.***

Keywords: Clones, seeds, fruits, time slot, crossing

1. Introduction

Yam belongs to the family Dioscoreaceae, and to the genus Dioscorea. It is a monocotyledonous plant that nevertheless has specific characteristics of a dicotyledonous plant (Degras, 1993). Yam is grown on all continents, mainly in Africa and the tropics where it is the staple food of more than 300 million people (FAOSTAT, 2018). *Dioscorea. rotundata* and *Dioscorea.alata* are the two main cultivated species (Ayensu and Coursey 1972).

In Côte d'Ivoire, yam is the leading food crop with an estimated volume of more than 7 million tonnes (Faostat, 2018). It is the staple food of a large part of the Ivorian population and is consumed in several forms: boiled, pounded, in stews and fried. The production of *D. alata* represents 55 to 60% of the total volume of yams. The Suidié varieties of the Bêtê-bêtê and Florido group represent more than 90% of the cultivated areas and are the best-selling on the market for *D. alata* (Doumbia et *al*., 2006, Kouakou, 2010).

Despite this importance, yam cultivation is facing some difficulties. Traditional varieties that are generally appreciated by consumers because of their good culinary qualities are disappearing. Some are susceptible to diseases such as anthracnose, others are depreciated because of the presence of blackheads in the tuber. (Kouakou, 2010, Doumbia and *al*., 2014). This is the case of the Suidié variety of the Betê-betê group, which tuber quality is altered by blackheads or "Internal Brown Spot" which is an IBS viral disease (Thouvenel and *al*, 1985, Girardin, 1996; Kouakou, 2010). In the market, the digitized and rough appearance of tubers of some varieties reduces their market values (Thouvenel and *al*., 1985). Except Florido, a variety introduced from Porto Rico and widely distributed and adopted by farmers, very few varieties have been developed and adopted (Kouakou and *al*, 2012). To face all these constraints, breeding for new varieties is an efficient way. to obtain varieties with good organoleptic qualities, tolerant to diseases and pests, and having beautiful shapes to facilitate harvesting, good conservation properties and adapted to market needs.

Breeding *D. alata* was rare until the 1990s (Abraham and *al*., 1986; Bai and Jos, 1986). Varietal selection was essentially limited to the mass selection within existing populations (Ettien and Tschannen., 2003; Kouakou and *al*., 2012). However, attempts to improve yam by crossing have been made worldwide (Abraham and. Nair, 1990; Abraham, 1992; Ano and *al*., 2002; Egesi and Asiedu, 2002 Arnau and *al*., 2010, Lebot and *al*., 2019), but remain insufficient due to lack of fertile of the parents Indeed, the primary constraint to the success of manual crossing in this species is the low number of flowering cultivars. Studies carried out in India and Vanuatu, two centres of diversification of this species, have shown that among the genetic resources existing in these regions only 10% were flowering (Abraham., 1997; Lebot and *al*., 2019). In Côte d'Ivoire, only 13 out of the 93 accessions existing in the collection were flowering (Kouakou and *al*., 2007). In this species, dioecium also acts as a hindrance on pollination and genetic improvement. In fact, male and female flowers are carried by different clones, which makes difficult flowers of the opposite sex to

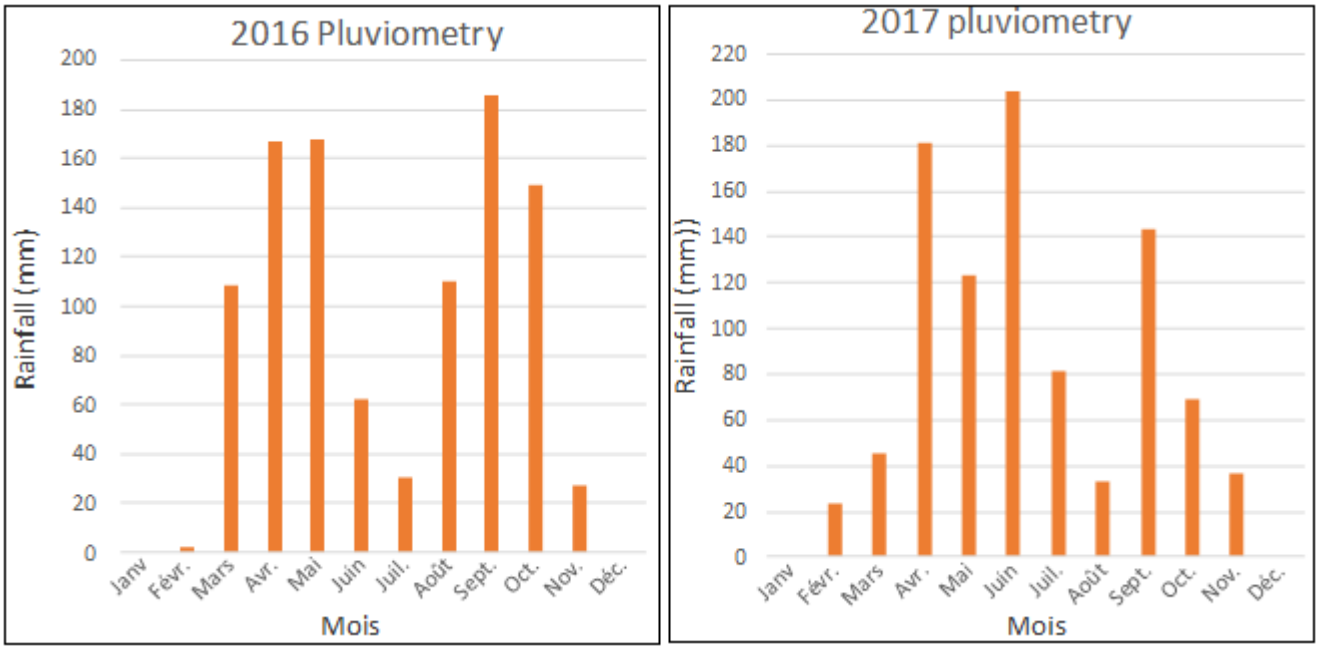
spontaneously meet. Pollination is entomophilic but ineffective because of the stickiness of pollen grains (Sadik and Okereke,1974, Akoroda, 1983; Degras, 1986; Abraham and Nair, 1990; Zoundjihépkon, 1993; Gamiette, 1999). Polyploidy is also a limiting factor in crossbreeding in *D. alata*. Indeed, there are accessions in this species with three different levels of ploidy (2n= 40, 60, 80 = 2x, 3x, 3x, 4x) and some combinations can lead to the formation of non-viable gametes or abnormal development of the endosperm (Arnau et *al*., 2011; Nemorin et *al*., 2013). Finally, the synchronization of male and female flowers is a major disadvantage to the success of crosses. Male and female blooms are not always in synchronous (Abraham and Nair, 1990; Norman et *al*., 2018; Lebot et *al.*, 2019), especially since the sex ratio (3:1) is disequilibrated with a predominance of male plants (Martin and Cabanillas, 1966; Degras, 1986; Abraham and Nair, 1991; Malapa, 2000, Sartie and Asiedu, 2014). Flowering time in male clones is between 12 and 20 days and in females between 5 and 10 days (Abraham and Nair., 1990). In India, Abraham's observations showed that male flowers open around 12 noon and pollen was viable for 4 hours (Abraham and Nair., 1990). Since pollen does not reach full maturity before 12 noon, pollens from these early morning flowers would not be mature and therefore not viable. To verify this hypothesis, a pollination time slot was tested by taking into account the opening time of the male flowers (7:30 in the morning) until the pollen viability time (4 h). Thus, pollination was carried out early in the morning at the opening of the male flowers (7:30 in the morning) until 11:00 am. This time called H1 was compared to the time between 11am and 2pm (H2). In Côte d'Ivoire, because of the limited success of the manual crossing, the yam genetic improvement program have been based for a long time on the open pollination of a small quantity of females Recently, as part of the Africayam regional project to develop yam improvement work in West Africa to improve income and food security, some flowering male and female clones have been identified and characterized in the collection of the Centre National de Recherche Agronomique (CNRA) for use as breeding animals. The objective of this study was to produce true seeds of *D. alata* by manual cross pollination.

2. Materials and method

2.1 Presentation of the study site

The field work was carried out in Bouaké in the center of Côte d'Ivoire, at the Food Crops Research Station (Station de Recherche sur les Cultures Vivrières) of the Centre National de Recherche Agronomique (CNRA) located at 7°40' N and 5°2' W. The climate is of the Baoulean type and the rainfall regime is bimodal with two rainy seasons from April to July and September to October, and two dry seasons in August and from November to March. The soils on this station are ferralitic, gravel, reworked, not deep and derived from a granitic alteration material (Kouamé, 1992). The annual rainfall is 1011.6 mm in 2016 and 939.7 mm in 2017 (Graph 1).

Rainfall data for both years are presented in the graphs below.



Graph 1: Rainfall at the Food Crops Research Station of Bouaké in 2016 and 2017

2.2 Planting material

Twelve clones of *D. alata* including five females (OA49, TDa01/00018, TDa01/000295, TDa99/00240, TDa01/00003) and seven males (23, 23bulbille, TDa00/00095, TDa01/00128, TDa01/00113, TDa01/0000004 and Ma01) were used for crosses. These materials were from the CNRA in vivo yam. The table below shows their characteristics.

Table 1: Morphological and quality characteristics of the varieties used

Clones	Type of clones	Sex	Flowering status	Quality		Resistance/Tolerance to Anthracnose
				Morphology of tuber	Boiled and pounded yam	
OA49	landrace	F	few	rough, irregular	bad	susceptible
TDa01/00003	hybrid	F	full	smooth, elongated	bad	resistant
TDa01/00018	hybrid	F	full	smooth, elongated	good	tolerant
TDa01/00295	hybrid	F	full	smooth, elongated and digitized	bad	tolerant
TDa99/00240	hybrid	F	full	smooth, elongated and digitized	bad	tolerant
Ma01	landrace	M	peu	smooth, rounded,	good	susceptible
23	landrace	M	medium	rough, elongated	good	tolerant
23bulbille	landrace	M	medium	smooth, rounded, and digitised	good	tolerant
TDa00/00128	hybrid	M	full	smooth, elongated and digitized	bad	tolerant
TDa00/00095	hybrid	M	full	rough, elongated and digitized	good	sensitive
TDa01/00113	hybrid	M	full	smooth, elongated and digitized	good	tolerant
TDa01/00004	hybrid	M	full	smooth, elongated and digitized	good	tolerant

F : female ; M : male ;

3. Methods

3.1 Device for setting up manual crossing blocks

The female and male parents were planted on 2 different blocks. The male and female blocks were separated by at least 500 m. The female block was arranged in a randomized complete block design with three replications and each female clone consisted of 60 plants per replication. The male block was established in a single block without repetition and each male clone consisted of ten plants arranged on a ridge. On the same ridge, the plants of the same clone were separated by 2 m.

To have a synchronization of male and female flowering, two planting dates were adopted. The male clones were planted two weeks and four weeks after the female block. An automated irrigation system was used on the female block to compensate the lack of water during the dry period, which generally begins at the end of October to the beginning of November. Depending on the soil moisture, irrigation took place two to three times a week during 4 hours.

3.2 Manual pollination

In 2016, pollination was carried out from October 12th to November 18th and from October 16th to November 24th in 2017. Two time schedules were tested: H1 between 7:30 am and 11 am and H2 between 11 am and 2 pm. Maturity of female flowers was detected by the opening of the first flowers at the base of the inflorescence. The inflorescence was then bagged to avoid any contamination. When most bagged female flowers were open, pollination was carried out. The mature male flowers are well individualized and light green. At this stage, these flowers were harvested and then taken to the female block. At maturity, the anther which is pale yellow or chick yellow and often sticky was removed with a needle and placed on the open stigmas (Fig. 2b). The inflorescence was then protected with a cloth bag for 2 weeks (Fig. 2c). After this period the pollination bag was removed. The evolution of the fruits was followed until the senescence of the plants.

3.3 Determination of the ploidy level of the parents

Cytometric analyses to determine the ploidy levels of male and female parents were carried out at the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) in Guadeloupe. Samples of fresh leaves from each clone were collected as described by Malapa *et al* (2005). The analysis were carried out on adult green leaves according to the protocol described by Arnau *et al* (2009 modified). To isolate and colour the nuclei 0.5 cm² of leaves were chopped with the same amount of leaves from an internal control using a double-edged razor blade in a petri dish containing 0.5 ml of extraction buffer (0.14 M NaCl, 0.003 M KCl, 0.012 M NaH2PO4, 0.002 M KH2PO4, 0.005 M Na2SO3, 0.25 % triton X-100, pH 7.4). The suspension was then filtered through a 30 µm nylon filter. Approximately 150 µl of solution was recovered and mixed with 150 µl of extraction buffer with propidium iodide added (200 µg/ml). The suspensions were then incubated for about 5 minutes at room temperature. After incubation, the fluorescence of the nuclei was measured using a Beckman Coulter FC500 cytometer (Beckman-Coulter, Villepinte, France). The ploidy level was determined by comparing the fluorescence intensity peaks of the sample and the control Gamiette *et al*., (1999). A diploid cultivar (74F) of the species *D. alata* was used as a control.

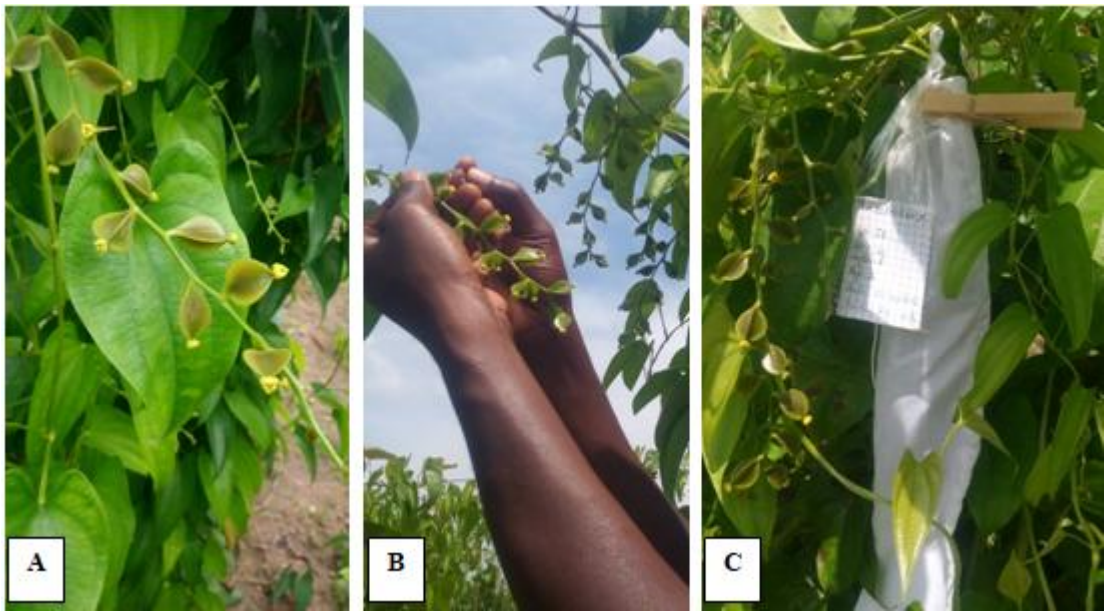


Figure 2: The steps of pollination : **a** : female flowers of TDa99/00240 ; **.b** : Pollination of TDa01/00003; **c** :TDa99/00018 flowers pollinated and bagged

3.4. Statistical analysis

The parameters measured by combination of parents are: the number of fruits formed, the number of seeds obtained, the pollination efficiency (% fruits) and the seed rate

Pollination efficiency (% fruits) = (Number of fruits formed x100) / Number of pollinated flowers)
Seeds rate (% seeds) = (Number of seeds x100) / (Number of fruits formed x 6)
Seeds rates (% seeds) was calculated as number of seeds devised number of fruits formed; where the number of seeds was taken to be 6 x number of fruits (Abraham et *al.*, 1990).

The data were analyzed with XLSTAT software version 19.03.44616, the statistical tests performed were Duncan tests (for peer mean comparison) and analysis of variance (ANOVA) for multiple comparison groups. A p-value of 0.05 was considered statistically significant.

4. Results

4.1 Ploidylevel of the parents

Flow cytometric analysis showed 2 levels of ploidy: 11 diploids and 1 triploid .(Table 2).

Table 2: Origin and ploidy levels of parents *D. alata*

Clones	Origin	Sex	Ploidy
23 bulbille	India	Male	2x
TDa01/00113	IITA (Nigeria)	Male	2x
TDa00/00128	IITA (Nigeria)	Male	2x
TDa01/00003	IITA (Nigeria)	Male	2x
23	India	Male	2x
TDa00/00095	IITA (Nigeria)	Male	2x
TDa01/00004	IITA (Nigeria)	Female	2x
TDa01/00018	IITA (Nigeria)	Female	2x
TDa99/00240	IITA (Nigeria)	Male	2x
MA01	Martinique	Female	2x
TDa01/00295	IITA (Nigeria)	Female	2x
OA49	Porto Rico	Female	3x

4.2 Number of fruits and seeds formed per crossing during the two years

Manual pollination in both years lasted between 38 and 40 days. During this period, all male and female clones were receptive. A total of 22987 crosses were carried out during the two years (13681 in 2016 and 9306 in 2017) corresponding to 33 parental combinations. (Table 3). Fruit and seed rates obtained in 2016 and 2017 were not statistically different. In the first year, from 13681 flowers pollinated, 3597 fruits and 6392 seeds were harvested, for a rate of 25.06% and 30.05% respectively. In the second year, from 9 306 flowers pollinated 2124 fruits and 3426 seeds were obtained, i.e. rates of 22.82% and 30.18% respectively.

Although fruiting and seed rates were not statistically different between the two years, there were some variations among these rates from one year to the next for some crossing pairs. Thus, the fruiting rates of the female TDa01/00003 crossed with 23bulbille, TDa00/00095, TDa00/00128 males respectively during the first year (4.9%; 34% and 50.63%) were lower than those of the second year (25.20%; 80.88% and 97.73%). However, seed rates in both years were statistically identical. Concerning the crossing of this female TDa01/00003 with the male TDa01/00004 it gave higher fruiting and seed rates in the first year (56.33% and 45.2%) than those obtained in the second year (29.21% and 14.89%). Fruiting and seed rates obtained by crossing the female TDa01/00018 with the male TDa00/00128 were higher in the first year (41.04% and 17.27%) than in the second year (19.37% and 3.49%). This same female TDa01/00018 crossed with TDa00/00095 and TDa01/00113 respectively gave higher fruiting rates in the first year (30.31% and 47.13%) than in the second year (20% and 28.15%); but lower seed rates in the first year (21.07% and 18.28%) than in the second year (43.21% and 22.92%). In addition, the crossing of the female TDa99/00240 with the male TDa00/00128 gave lower fruiting rates in the first year (22.9%) than in the second year (72%) but lower seed rates in the second year (1.16%) than in the first year (17.78%).

This same female TDa99/00240 crossed with TDa00/00095 and TDa01/00113 respectively gave higher fruiting rates in the first year (32.68% and 43.4%) than in the second year (6.22% and 8.19%); but lower seed rates in the first year (26.37%, 37.32%) than in the second year. (55.56%, 42.86%). Finally, the female clone TDa01/00295 crossed with the males 23bulbille and TDa00/00128 gave higher fruiting rates in the first year (26.94%; 49.39%) than in the second year (19.52%, 32.95%) but lower seed rates in the first year (8.75%; 12.95%) than in the second year (19.15%, 34.48%).

4.3 Effect of male and female parents on fruiting and seed rate

The average fruiting rates were 20.02% in 2016 and 27.38% in 2017, and the seed rates were 27.91% in 2016 and 31.15% in 2017. The overall averages of fruiting and seed rates were 23.7% and 29.53% for both years. (Table 4). During the first year, the fruiting rate ranged from 2.69% to 32.89% respectively for OA49 and TDa01/00003 for all the seven crosses where they were involved and the seed rate from 4% to 41.39% respectively for OA49 and TDa01/00003. During the second year, the mean fruiting rate were 0% for OA49 and 35.06% for TDa01/00003 for all the crosses for which they were used as females and the seed rate were 0% and 32.81% for the same females. OA49 had the worse fruiting and seeding rates among all females in both years and the TDa01/00003 female had the highest rates.

As far as the male clones are concerned, 23 and Ma01 crossed with all the females gave the lowest fruiting rates during the first year. This rate was 0% for OA49x23 and 14.55% for TDa01/00003x23. Clone 23 crossed with TDa99/00240, TDa01/00295 and OA49 respectively gave very low fruiting rates (0.48%, 0.56% and 0%) and zero seed rates (0%). The Ma01 clone crossed with TDa01/00003 yielded fruiting and seed rates of 1.45% % and 16.67% in 2016 compared to 9.09% and 8.33% in 2017. Analysis of variance have shown that there is a significant difference between female clones for the fruiting rate at the 5% threshold (Table 5). The Duncan mean comparison test established two groups of clones. The first group gathered mainly hybrid clones with comparable averages and the second group formed by the clone OA49. The analysis of variance also showed that there is a significant effect of male parents on the fruiting rate at the 5% threshold (Table 5). Duncan's test revealed three different groups. The first group formed by TDa00/000128 which had the higher average fruiting rate (48.25 %) than the others. The second group consisting of the clones TDa00/00095, TDa01/00004 and TDa01/00113 with lower average fruiting rates than the first group. The third group including clones 23 and Ma01 with very low average fruiting rates (2.04 %). There is an intermediate group between groups 2 and 3 containing only the clone 23bulbille (18.93 %). There is no significant difference at the 5% threshold for either female or male clones on the seed rate (Table 4).

Table 3: Number of pollinated flowers, fruiting and seed rates by parental combination in *D. alata* in 2016 and 2017

Parental combinations		2016					2017				
		Flowers	Fruits		Seeds		Flowers	Fruits		Seeds	
Females	Males		no	%	no	%		no	%	no	%
TDa01/00003	23	349	2	0.57	6	-		-	-	-	-
	23Bulbille	143	7	4.9	21	-	127	32	25,20	75	39,06
	TDa00/00095	403	137	34	292	35.52	387	313	80,88	632	33.65
	TDa01/00004	1184	667	56.33	1809	45.2	1253	366	29.21	327	14.89
	TDa00/00128	559	283	50.63	600	35.34	132	129	97.73	297	38.37
	TDa01/00113	701	154	21.97	387	41.88	943	162	17.18	459	47.22
	Ma01	482	7	1.45	7	-	22	2	9.09	1	-
	Total	3821	1257	32.89	3122	41.39	2864	1004	35.06	1791	29.73
TDa01/00018	23	55	8	14.55	0	-		-	-	-	-
	23Bulbille	110	24	21.82	27	18.75	255	31	12.16	31	16.67
	TDa00/00095	574	174	30.31	220	21.07	405	81	20.00	210	43.21
	TDa01/00004	1187	210	17.69	131	10.4	1472	341	23.17	404	19.75
	TDa00/00128	402	165	41.04	171	17.27	222	43	19.37	9	3.49
	TDa01/00113	505	238	47.13	261	18.28	341	96	28.15	132	22.92
	Ma01	287	16	5.57	0	0					
	Total	3120	835	26.76	810	16.16	2695	592	21.97	786	22.13
TDa99/00240	23	442	1	0.23	0	-	415	2	0.48	0	-
	23Bulbille	693	150	21.65	540	60	62	22	35.48	28	21.21
	TDa00/00095	205	67	32.68	106	26.37	193	12	6.22	40	55.56
	TDa01/00004	843	341	40.45	394	19.26	362	134	37.02	395	49.13
	TDa00/00128	131	30	22.9	32	17.78	100	72	72.00	5	1.16
	TDa01/00113	818	355	43.4	795	37.32	171	14	8.19	36	42.86
	Ma01	372	2	0.54	2	-					
	Total	3504	946	26.99	1869	32.92	1303	256	19.65	504	32.81
TDa01/00295	23	357	0	0	0	-	900	5	0.56	9	-
	23 Bulbille	219	59	26.94	31	8.75	584	114	19.52	131	19.15
	TDa00/00095	110	12	10.91	42	58.33					
	TDa01/00004	573	213	37.17	184	14.4	287	105	36.59	53	8.41
	TDa00/00128	245	121	49.39	94	12.95	88	29	32.95	60	34.48
	TDa01/00113	701	129	18.4	244	31.52	102	19	18.63	92	80.70
	Ma01	102	0	0	0	-					

	Total	2307	534	23.14	595	18.57	1961	272	13.87	345	21.14
OA49	23	51	0	0	0		483	0	0		
	23 Bulbille	445	12	2.7	6	8		-			
	TDa00/00095										
	TDa01/00004	230	13	5.65	0	0					
	TDa00/00128					0					
	TDa01/00113	142	0	0	0	-					
	Ma01	61	0	0	0						
		929	25	2.69	6	4	483	0			
	Total	13681	3597	25.06	6392	30.05	9306	2124	22.82	3426	30.18

Table 4: Average means of fruit and seed setting on two years

		2016-2017		2016	2017	2016	2017
Females	Males	% Fruits	% Seeds	% Fruits		% Seeds	
TDa01/00003	7	33.01 ± 8.40a	36.79 ± 3.13a				
TDa01/00018	7	21.64 ± 3.59a	19.18 ± 3.38a				
TDa99/00240	7	24.71 ± 5.96a	33.07 ± 5.99a				
TDa01/00295	7	20.87 ± 4.70a	29.85 ± 8.30a				
OA49	7	1.67 ± 1.12b					
Males	Females						
TDa00/00128	5	48.25 ± 9.24a	20.11 ± 5.12a				
TDa01/00004	5	31.47± 4.88b	22.68 ± 5.52a				
TDa00/00095	5	30.71 ± 9.30b	39.10 ± 5.31a				
TDa01/00113	5	22.56 ± 5.06b	40.33 ± 7.54a				
23Bulbille	5	18.93 ± 3.53bc	26.23 ± 6.76a				
23	5	2.04 ± 1.79c					
Ma01	5	2.77 ± 1.52c					
Mean				20.02	27.38	27.91	31.15
Mean overall				23.7			29.53

The percentages followed by the same letters are not significantly different (P ≤ 0.05)
7: all male varieties; 5: all female varieties

4.4 Variation of the fruit setting rate according to the time of pollination

In 2016, the time slot effect is significant ($p < 0.05$) on the fruiting rate for the male TDa00/00095. For this male, the fruiting rate obtained at the first time slot is significantly higher than that obtained at the second time slot. For all the other males, the time slot has no effect on the fruiting rate. The fruiting rates obtained at the two schedules H1 and H2 are statistically comparable.

Table 5: Influence of the male clone on the fruiting rate according to the time slot

		Flowers pollinated		Fruits setting			
Mâles	Femelles	H1	H2	No (H1)	No (H2)	% H1	% H2
TDa01/00113	TDa01/00003	412	289	96	58	23.30	20.07
	TDa01/00295	422	279	78	51	18.48	18.28
	TDa01/00018	263	242	123	115	46.77	47.52
	TDa99/00240	489	329	246	109	50.31	33.13
	Total	*1586*	*1139*	*543*	*333*		
	Mean	*396.5*	*284.75*	*135.75*	*83.25*	34.71a	29.75a
TDa01/00004	TDa01/00003	671	513	428	239	63.79	46.59
	TDa01/00295	444	129	159	54	35.81	41.86
	TDa01/00018	683	504	99	111	14.49	22.02
	TDa99/00240	464	379	239	102	51.51	26.91
	Total	2262	1525	925	506		
	Mean	565.5	381.25	231.25	126.5	41.4a	34.35a
TDa00/00095	TDa01/00003	264	139	100	37	37.88	26.62
	TDa01/00018	338	236	123	51	36.39	21.61
	TDa99/00240	101	104	39	28	38.61	26.92
	Total	703	479	262	116		
	Mean	234.33	159.66	87.33	38.66	37.63a	25.05b
TDa00/00128	TDa01/00003	372	187	189	94	50.81	50.27
	TDa01/00295	123	122	70	51	56.91	41.80
	TDa01/00018	294	108	92	73	31.29	67.59
	TDa99/00240	65	66	8	22	12.31	33.33
	Total	*854*	*483*	*359*	*240*		
	Mean	*213.5*	*120.75*	*89.75*	*60*	37.83a	48.25a
23bulbille	TDa01/00003	87	56	0	7	0.00	12.50
	TDa01/00295	177	42	59	0	33.33	0.00

	TDa99/00240	364	329	94	56	25.82	17.02
	Total	*628*	*427*	*153*	*63*		
	Mean	*209.33*	*142.33*	*51*	*21*	19.72a	9.84a

The means with different letters are significantly different at p <0.05. H1 (7:30-11am) and H2 (11-14pm).

5. Discussion

Number of fruits and seeds formed per crossing pair during the two years

The overall averages of fruiting (23.7%) and seeding (29.53%) rates obtained in this study over the two years are very important given the difficulty of pollination and the generally low success rates. In natural pollination, Abraham (1989) showed that the fruiting rate was very low (0.2- 3%). These rates show that the parents used in our crosses have a high potential for fruit and seed production. The fruiting rates obtained in this study are significantly lower than those obtained by Lebot et *al*, (2019) by crossing two improved clones IN(2X) x IN(2X) and one improved clone IN(2X) with a cultivar VU(2X). In contrast, seed rates are statistically identical. These rates could increase if weather conditions are more favourable.

The overall mean of fruit setting was higher for 2016 than that of 2017. A decrease was observed for 12 crossing pairs between 2016 and 2017 whereas this number was increasing for 7 crossing pairs. The decrease in fruiting rate could be explained by the decrease in rainfall during 2017 pollination. Indeed, rainfall data for both years show that the amount of rainfall from October to November in 2016 (1011.6 mm) was higher than in 2017 (939.7 mm) for the same period. This may have influenced the fruit setting. Indeed, according to Abraham and Nair (1990), a high relative humidity and a decrease in temperature during the pollination period would be more conducive to fruit setting. Another explanation for the observed changes in fruiting rate in some crossing couples may be related to the fertility of the female clone. Indeed, in the female TDa01/00003 the increase observed in 2017 in couples TDa01/00003x23bulbulbulbille, TDa01/00003xTDa01/00095, TDa01/00003xTDa01/00128 could be due to a variation in its-fertility because, according to Norman *et al* (2018), flower fertility is an important factor which could influence fruiting rates. This variation in fertility according to Akoroda (1982) is believed to be due to climatic conditions (Akoroda, 1982; Martin 1963). Indeed, in *D. alata*, female clones flower from September to October. During this period, the absence or lack of rain could affect the receptivity of the forming flowers and therefore reduce the seed rate. Some crossing couples such as TDa01/00003xTDa01/0000004 and TDa01/00018xTDa00/00128 have higher fruiting and seed rates in 2016 than in 2017. This decrease in rates could be explained by a decrease in male clone fertility (Sadik and Okereke, 1974; Akoroda, 1982). This suggests that in some male clones, fertility may vary from year to year or even remain constant depending on various environmental or climatic factors. Colouring pollen with carminoacetic acid may be a good way to confirm this (Abraham, 1992). Some couples give a lot of fruit but very few seeds. This is the case for the pairs TDa99/00240xTDa00/00128 and TDa01/00295x23bulbulbille which gave high fruiting rates but very low seed rates. The low seed rate may be due to post-zygotic hybridization barriers as observed in some species such as potatoes (Erazzu *et al*., 1998), and sweet potatoes (Martin and Cabanillas 1968). Indeed, after fertilization, the female flower turns into a fruit. During this formation, the endorsperm nourishing tissue plays an important role in the development of the seed because, an abnormal development of the seed systematically causes the rejection of the embryo. In the case of yams, fertilization would lead to the formation of swollen fruits and then post-zygotic barriers due to an imbalance in the 2:1 ratio between maternal and paternal cells (Johnson *et al*., 1980; Némorin, 2012). Consequently, there follows a malformation of the endosperm, then an abortion of the embryo and finally a malformation of the seed, hence the very low seed rates observed in these crossing pairs.

Effect of male and female parents on fruiting rate

Fruiting and seed rates of the female OA49 were extremely low in 2016. In 2017, crosses made on this female did not yield any fruit. Cytometric analyses performed showed that this female is triploid Nemorin *et al* (2013) and Lebot *et al*., (2019) indicated that it is not possible to obtain viable seeds from crosses where one of the progenitors is triploid. The other females are all diploids and hybrids. Indeed, diploid x diploid crosses have a higher probability of producing fruit with viable seeds. In addition, the very high fertility of hybrids is believed to be due to the fact that they are derived from seeds (Arnau *et al*., 2010). Indeed, according to these authors, the use of seeds resulting from manual crossing or controlled pollination between low-fertility breeders makes it possible to obtain very fertile hybrids. This fertility is generally reflected in high flowering intensity due to hybrid vigour (Sadik, and Okereke, 1974; Akoroda, 1982, Norman *et al*., 2018) and high fruiting rates. A low fruiting and seed rates were obtained in both years by crossing the male Ma01 and 23 clones which are landraces with all females.These clones had also a low flowering intensity and smaller anthers, which would result in a significant decrease in fruit and seed rates (Norman et al., 2018). Another explanation for this phenomenon is the very low fertility of these males due to a high rate of very low fertility pollens (Sadik, and Okereke, 1974; Akoroda, 1982). Thus, in *D. rotundata*, Sadik and Okereke's (1974) showed that the low fruiting rate observed in this species was due to the very low fertility of pollen. Abraham (1992), observed these two phenomena in various cultivars of *D. alata*.

For male clones, the ANOVA analysis resulted in an effect on the fruiting rate. Groups 1 and 2 formed by hybrid clones are more fertile than group 3 consisted by landraces. However, clone 23bulbille obtained from bulbil had crossed with some hybrid female clones performed like the crosses between hybrids for fruiting rates. In conclusion, hybrid clones and clones deriving from bulbil are more fertile than landraces. As far as the fruit set is concerned, there was difference whether the parents are hybrids or not.

Effect of time slot on fruiting rate

This study showed that there is a time slot effect on the fruiting rate only for the male TDa00/00095. For this male, the fruiting rate is higher at the H1 schedule than at the H2 schedule. For this parent, we noticed that the flowers freshly harvested at the H1 schedule have the most sticky pollen. This pollen adheres more easily to the surface of the stigma and thus increases the chances of germination. At the H2 time, when the sunshine is more intense, some male flowers start to turn black, and the pollen becomes less and less sticky. It can therefore fall under the effect of the wind. Since female flowers in *D. alata* have the stigma surface oriented towards the soil, pollens with little or no stickiness reduce their chance of germination, reducing the rate of fruiting. This clone could therefore have a more early maturation of the pollen and a shorter fertility time. For other males, there is no hourly effect on the fruiting rate. For these males, pollination at both times gave comparable results. In India, Abraham (1990) revealed that pollinations executed between 12pm and 3pm gave high fruiting rates because, according to him, the opening of the male flowers around 12pm ensured a good fertility. This study proved that pollinations carried out between 11am and 2pm also give high fruiting rates. It means that the opening of the male flower can happen before 12pm and the pollen remain fertile till 2pm as written.

6. Conclusion

This study, conducted over two consecutive years for the production of hybrid seeds by manual crossing, produced fruits and seeds from several crossing pairs. Fruiting rates depend on the male parents used and their level of ploidy, because among these male parents, hybrids have proved to be more fertile than landraces. This study also showed that fruiting rates according to time slot where pollination was made were influenced by the male parent. For the majority of the males, the average fruiting rates at both crossing schedules were statistically identical However, the seed rates obtained were not dependent on the parents but could rather result from phenomena related to environmental or climatic conditions or cytogenetic phenomena. However, in the dynamics of varietal creation, it would be interesting, on the one hand, to prefer crosses between hybrids as parents and, on the other hand, to carry out pollination between 7:30 am and 11 am.

Very little work has been done on pollination among *D. alata* due to plant difficulties. However, the fruiting and seed rates obtained on this study show that the technique is well controlled. Therefore, breeding *D. alata* for prior traits can be realized

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8. Author Contribution

AEE participated in the design of this study, performed the crossings, collected and analyzed the data and wrote the manuscript. AMK participated in the design of this study and to the correction of the manuscript. JCN participated to the correction of the manuscript. KEBD participated in the design of this study, performed the crossings and participated in the correction of the manuscript. YB participated in the design of this study and data collection. BSE participated to the correction of the manuscript. NB participated to the correction of the manuscript. AG participated in the data analysis, wrote the manuscript. EM participated to the correction of the manuscript. AA participated in the correction of the manuscript. PA participated in the correction of the manuscript. ASPN participated in the design of this study and in the correction of the manuscript.

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10. Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

11. Conflict of interest

The authors declare that they have no conflict of interest.

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