PLOIDY STATUS OF *Dioscorea alata* L. AND ITS RELATIONSHIP WITH RESISTANCE TO ANTHRACNOSE IN CÔTE D'IVOIRE

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ABSTRACT

Dioscorea alata L. is the most cultivated yam species in Côte d'Ivoire. It rarely flowers and, as a result, is propagated through vegetative means. Fruit setting is not common because of the variation in ploidy within species. Anthracnose is the main desease of *Dioscorea alata* L. Flow cytometry was used to estimate the ploidy level of 43 accessions of *D. alata*. Susceptibility of these accessions to Anthracnose, the main disease on the tuber, caused by a fungus (*Colletotrichum gleosporioides*) and flowering abilities investigated and five ploidy levels were accessed. Sixty seven percent of the studied genotypes were found not susceptible to Anthracnose. Of the susceptible ones, only 5 % were severely damaged. Sixty-nine percent of the accessions did not flower. All male accessions (19 %) were tetraploid, whereas female (12 %) were either hexaploid (80 %) or tetraploid (20 %). Flowering clones were found to be not susceptible to Anthracnose.

Key-words : Anthracnose, *Dioscorea alata*, flow cytometry, ploidy level, yam, Côte d'Ivoire.

RESUME

RELATION NIVEAU DE PLOÏDIE ET SENSIBILITE A L'ANTHRACNOSE CHEZ Dioscorea alata L. *EN CÔTE D'IVOIRE*

Dioscorea alata L. *est l'espèce d'igname la plus cultivée en Côte d'Ivoire. Son aire de culture couvre la quasi-totalité du territoire. C'est une des plus importantes plantes alimentaires. Les ignames sont reproduites par voie végétative et la floraison est rare. La fructification est rare à cause de la variation du niveau de ploïdie au sein des espèces et des facteurs environnementaux. L'anthracnose est la principale maladie chez l'espèce. La cytométrie en flux a été utilisée pour estimer le niveau de ploïdie de 43 accessions d'igname de* D. alata. *La sensibilité à l'anthracnose causée par* Colletotrichum gleosporioides *et la floraison ont aussi été étudiées. Cinq niveaux (3,5x ; 4x ; 4,5x ; 6x et 8x) ont été décelés. Soixante sept pourcent (67 %) des génotypes étudiés n'ont pas été sensibles à l'anthracnose. Parmi les clones sensibles seulement 5 % ont subi une attaque sévère. Soixante neuf pourcent des accessions n'ont pas fleuri. Toutes les accessions mâles (19 %) sont tétraploïdes tandis que les femelles (12 %) sont, soit hexaploïdes (80 %), soit tétraploïdes (20 %). Les clones florifères n'ont pas été sensibles à l'anthracnose.*

Mots clés : *Anthracnose,* Dioscorea alata*, cytométrie en flux, niveau de ploïdie, igname, Côte d'Ivoire.*

INTRODUCTION

Dioscorea cayenensis-Dioscorea rotundata complex and *Dioscorea alata* L. are the main cultivated yam species in Côte d'Ivoire. The former originated from West Africa, whereas the second was introduced to Africa. *Dioscorea alata* is the most widespread yam species in the world. No wild *D. alata* is known and its related species *D. permisilis* and *D. hamiltonii* are unsettled (Lebot, 2002). Its origin is not well known. It is said to come from South East Asia, Indonesia, West Indies or Indian Ocean (Burkill, 1935 ; Coursey, 1967 ; Martin et Rhodes, 1977). Despite its poor cooking quality, *D. alata* is preferred to the other yams, thanks to a high yield potential and a good post-harvest storage ability (Coursey, 1967). In Côte d'Ivoire *D. alata* accounts for 60 % of the total yam production. During the last 25 years, its cultivation spreads from the central zone, the main production area, to the whole country.

Anthracnose is the most important disease of *Dioscorea alata* in Côte d'Ivoire. Most varieties, well appreciated for their good cooking qualities, are susceptible to the desease (Nicole *et al.,* 1994). Improving existing varieties by combining both tolerance to Anthracnose and good cooking qualities is necessary. Unfortunately, most *D. alata* varieties, with good cooking qualities, rarely flower and production of yam plantlets from true seeds is limited (Asiedu *et al.,* 1998). Propagation techniques have a high potential to satisfy the increasing demand. It is therefore, necessary to study the physiology of the reproduction and the cytogenetic behaviour of *D. alata*. Knowing the ploidy status of the existing genotypes is an important step in yam breeding programs. The advanced basic chromosomal numbers are variable accross continents. X = 9 and X = 10 in Africa, X = 10 in Asia and X = 9 in America. They are di, tetra, hexa and octoploïd (Zoundjihékpon *et al.,* 1990). The level of ploidy is variable between species and within the same species. Thus one has within the complex *D. cayenensis- D. rotundata* : cv Lokpa : 2 N = 6X = 54 ; Krenglè : 2 N = 4X = 40 ; Gnan of Côte d'Ivoire : 2 n = 6X = 60 ; Gnan of Benin : 2 N = 40. For *D. alata,* there is a series of polyploid going from 2n = 20 to 2n = 140 chromosomes, with all the possible intermediaries (Miège, 1952 ; Ramachandran, 1968 ; Hamon *et al.,* 1992). Indeed, yam being monoecious, this knowledge will allow to make the proper choice of the parents to cross for more chance of success. The individuals with the same level are easier to cross than those with different level of ploidy.

Ploidy is usually estimated by cytological chromosome counting (Martin and Ortiz, 1963, 1966 ; Zoundjihékpon *et al.,* 1990 ; Abraham, 1998). However, yam chromosomes counting is tedious because they are tiny and most often clumped together. Flow cytometry method has been used successfully on yam by Hamon *et al.* (1992), Gamiette (1999) and Egesi *et al.* (2002). It is a rapid and efficient method to estimate ploidy level. This technique is based on the fluorescence of stained nuclei of individual chromosomes.

The objective of this study was to determine using flow cytometry, the ploidy level of some accessions of *D. alata* and access their resistance level to anthracnose disease.

MATERIAL AND METHODS

PLANT MATERIAL

Forty three accessions of *D. alata* from the *in vitro* gene bank of Centre National de Recherche Agronomique, located at Adiopodoumé (Côte d'Ivoire) are used for the study. The accessions were collected from different countries : Côte d'Ivoire, Cameroon, Togo, India, Puerto Rico, Martinique, New Caledonia and Brazil.

METHODS

Young leaves were collected from individual plants for flow cytometry analysis. About 1 cm^2 of leaf tissue was obtained with a sharp razor blade. Nuclei were released in a glass Petri dish containing 0.5 ml phosphate buffer (Dolozel *et al.,* 1989), modified with 10 % of triton). The homogenate was mixed and filtered through a 48 µm pore size nylon filter into a plastic tube "Becton Dickinson". Nuclei were stained adding an equal volume of propidium iodide into the suspension. Samples were then incubated for 1 to 5 mn at room temperature. The relative fluorescence intensity of stained nuclei was analysed using a Fascan Dickinson Analyser.

DNA index was estimated by analysing 3000 nuclei per sample. Three *D. alata* clones from Vanuatu (Vu 444, 2n = 4x ; Vu 760, 2n = 6x and Vu688, 2n = 8 x) were used as internal references.

The flow cytometer was adjusted so that tetraploid reference peaks was 200 nuclei. Calibration was periodically checked to minimise variation due to runs. Ploidy estimation was based on the following formula (1) by Gamiette, (1999) :

(1) NP = (I.F. / I.F.T.) x Ploidy of the reference ;

(NP = ploidy level ; I.F. = DNA index of the sample ; I.F.T. = DNA index of the internal reference).

Ploidy estimation was conducted at the INSERM laboratory of Montpellier, France.

Flowering and Anthracnose evaluation : Field trials were conducted at Bouaké in 2001 and Abidjan in 2003 in Côte d'Ivoire. Anthracnose severity was scored five months after planting as well as flowering ability using a 1 to 5 scale (Table 1) based on the necrotic area of the leaves and the portion of the vine attacked by the fungus. Ten plants were observed per accession. The highest level of infestation for the 2 years and the 2 locations was considered. The flowering ability was scored 0 for no flowering clone, 1 for male flowering only, 2 for only female flower and 3 for monoecious clone (both male and female flower). Ten plants were observed.

Table 1 : Scoring Anthracnose severity and flowering

Notation de la sévérité de l'anthracnose et de la floraison

Items	Stages	Score	Appreciation
Anthracnose severity	0 – 2 %	1	Not susceptible
	3-25 %	2	Not susceptible
	26 – 50 %	3	Susceptible
	> 50 %	4	Susceptible
	death Plant	5	Very susceptible
Flowering	No flowering	0	
	Male flowers only	1	
	Female flowers only	2	
	Monoecious	3	

RESULTS

PLOIDY LEVEL

Flow cytometry analysis revealed 5 levels of ploidy : 3.5x ; 4x ; 4.5x ; 6x and 8x (Table 2). Of the 43 accessions, only one (NC03) was octoploid, whereas 12 % were hexaploid and 61 % tetraploid (Figure 1). Ploidy levels of 3.5x and 4.5x were observed for 9 % and 16 % of the accessions, respectively (Figure 1). Within the 18 accessions from Côte d'Ivoire, one was 3.5x, 14 were 4x and 3 were 4.5x. Samples from Puerto Rico were 3.5x for 21 %, 4x for 36 %, 4.5x for 14 % and 6x for 29 %. Indian samples were 4x for 67 % and 4.5x for 33 %, whereas the accession from Martinique was tetraploid. The single octoploïd accession detected originated from New Caledonia (Table 2).

SEVERITY OF YAM ANTHRACNOSE

The severity of yam anthracnose varied from 1 to 4 on a 1 to 5 scale (Table 2). Of the 31 accessions observed, 94 % were not susceptible. None of the accessions died from anthracnose infection (Figure 2).

FLOWERING

The majority of the accessions (69 %) did not flower. The flowering accessions were mainly dioecious, with 19 % producing male flowers and 12 % producing female flowers (Figure 3).

RELATIONSHIP BETWEEN THE PLOIDY LEVEL, SEVERITY OF ANTHRACNOSE AND FLOWERING

Few tetraploid accessions were susceptible to yam anthracnose (8 %, Figure 4a). The severity of anthracnose was low for all hexaploïd

accessions : 20 % had score 1 and 80 % had score 2 (Figure 4b).

The Pearson Correlation Coefficient, calculated for ploidy level and anthracnose severity, was 0.609.

All hexaploïd genotypes flowered and the sex ratio was 80 % for female and 20 % for male (Figure 5a) whereas only 8 % of the tetraploid flowered (Figure 5b).

All the female accessions were scored 1 or 2 regarding anthracnose severity. All flowering clones were tolerant to anthracnose. The Pearson Correlation Coefficient, calculated for yam anthracnose severity and sex of flowering, was r = 0.609.

Table 2 : Ploidy level accessed through flow cytometry analysis of 43 *D. alata* accessions

Niveaux de ploïdie de 43 accessions de D. alata *déterminés par cytométrie en flux*

Origin	Accession	I. F.	I.F.T	Ploidy of the reference	Ploidy calculated (NP)	Estimated ploidy	CV
Puerto Rico	135	226.53	151.95	4	5.97	6	7.03
India	138	182.24	364.54	8	3.99	4	7.16
Côte d'Ivoire	169	145.70	152.99	4	4.2	4	7.22
Côte d'Ivoire	1832	188.43	173.27	4	4.35	4.5	5.7
Côte d'Ivoire	205	186.4	173.29	4	4.30	4.5	8.64
Côte d'Ivoire	208	245.21	467.36	8	4.2	4	10.59
Côte d'Ivoire	258	248.49	475.52	8	4.2	4	14.52
Togo	411	160	320	8	4	4	9.9
Côte d'Ivoire	655	154.53	173.19	8	3.59	3.5	6.53
Côte d'Ivoire	71	239.75	467.64	8	4.1	4	7.22
Côte d'Ivoire	890	200.63	394.94	8	4.06	4	18.16
Porto Rico	27B	182.82	397.77	8	3.67	3.5	21.16
Comores	AKE ASSI	240	160	4	6	6	6.99
India	BRAZO	190.88	173.56	4	4.4	4.5	7.16
Cameroon	C18	186.68	353.48	8	4.22	4	15.53
Côte d'Ivoire	DOUOKOKORE	228.04	463.04	8	3.94	4	15.09
India	EA12	165.08	165.58	4	4	4	4.03
Puerto Rico	FLORIDO	187.46	173.4	4	4.32	4.5	10.6
Puerto Rico	HAWAI	185.21	374.33	8	3.96	4	7.83
Côte d'Ivoire	IB16	211.62	423.7	8	3.99	4	19.08
Puerto Rico	IB21	220.31	379.95	8	3.48	3.5	14.31
Côte d'Ivoire	IB22	208.85	435.9	8	3.83	4	18.51
Puerto Rico	IB26	268.84	173.31	4	6.20	6	5.49
Côte d'Ivoire	IB27	199.04	411.46	8	3.87	4	16.81
Côte d'Ivoire	IB68	178.62	178.61	4	4	4	9.58
Côte d'Ivoire	IB88	250.15	476.46	8	4.2	4	15.35
Côte d'Ivoire	IB95	248.28	479.12	8	4.1	4	11.72
Martinique	MA02	182.94	173.21	4	4.22	4	8.19
Martinique	MA03	199.47	402.94	8	3.96	4	6.78
New Caledonia	NC03	361.63	185.72	4	7.72	8	6.15
Brazil	NE3SRT4	387.9	688.13	8	4.5	4.5	16.15
Puerto Rico	OA06	226.64	432.33	8	4.5	4.5	14.55
Puerto Rico	OA07	197.28	397.52	8	3.97	4	17.55
Puerto Rico	OA15	414.68	532.82	8	6.23	6	5.45
Côte d'Ivoire	OA44	205.24	205.24	4	4	4	7.4
Côte d'Ivoire	OA45	248.42	493.26	8	4.02	4	10.51
Puerto Rico	OA49	260.95	345.80	8	6.04	6	6.82
Puerto Rico	PR01	174.04	377.59	8	3.68	3.5	16.16
Puerto Rico	PR04	259.32	500.40	8	4.14	4	8.13
Puerto Rico	PR05	227.13	470.89	8	3.86	4	17.83
Puerto Rico	PR06	256.8	517.41	8	3.97	4	9.18
Côte d'Ivoire	SUIDIE	242.49	478.87	8	4.05	4	13.76
Côte d'Ivoire	TCHEKEDE	194.2	173.52	4	4.48	4.5	7.87

NP = (I.F./I.F.T.) x Ploidy of the reference ; NP = ploidy level ; I.F. = DNA index of the sample ; I.F.T. = DNA index of the internal reference; C V : Coefficient of variation

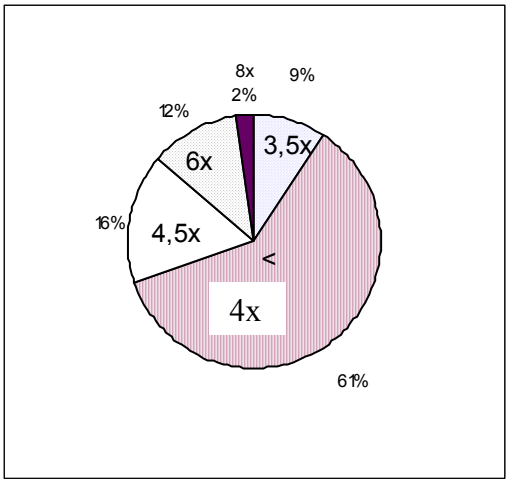


Figure 1 : Percentages of each level of ploidy within studied population of yams *D. alata*

Pourcentage de chaque niveau de ploïdie dans la population étudiée de D. alata

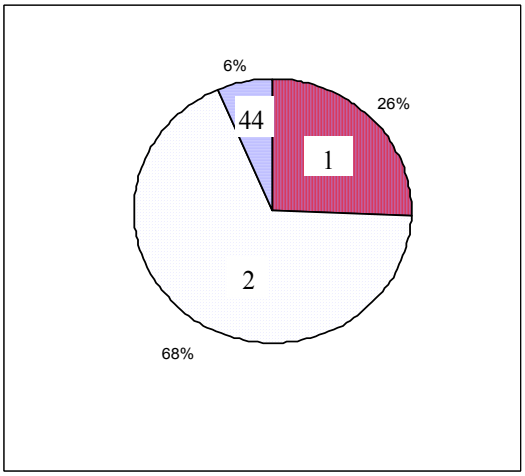


Figure 2 : Severity of anthracnose on 31 accessions of *D. alata*

Sévérité de l'anthracnose au sein de 31 accessions de D. alata

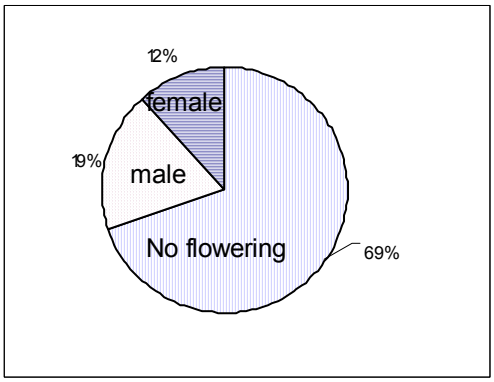
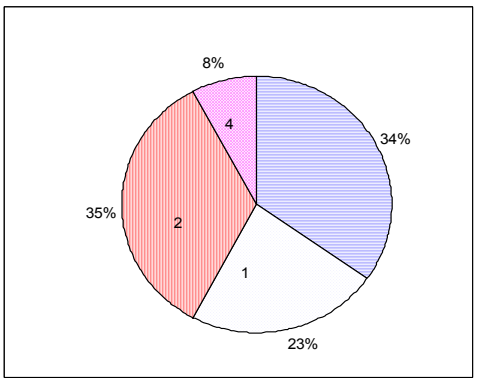
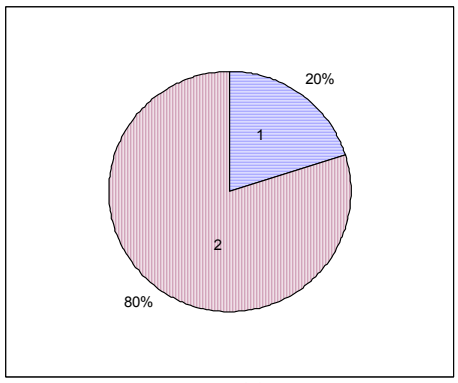


Figure 3 : Sex ratio within studied population of *D. alata*

Sex ratio au sein de la population étudiée de D. alata



a)



b)

Figure 4 : Anthracnose severity within the tetraploid (a) and hexaploid (b) populations of *D. alata*

Sévérité de l'anthracnose parmi les populations tétraploïdes (a) et hexaploïdes (b) de D. alata

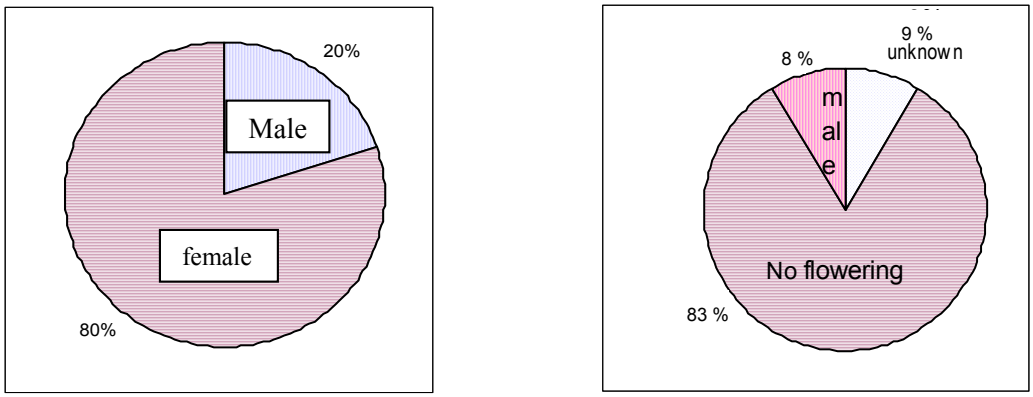


Figure 5 : Male and female flowers within hexaploid (a) and tetraploid accessions (b) of *D. alata*

Fleurs mâles et femelles au sein des accessions hexaploïdes (a) et tétraploïdes (b) de D. alata

DISCUSSION

The 5 ploidy levels revealed by flow cytometry analysis confirm the large variation of *D. alata* chromosome number reported by previous studies (Miège, 1952 ; Ramachandran, 1968 and Hamon *et al.,* 1992). These authors reported 30, 40, 50, 60, 70 and 80 chromosomes that gives levels of ploidy 3x, 4x, 5x, 6x, 7x and 8x among *D. alata.* Gamiette (1999) and Egesi *et al.* (2002) found only 4x, 6x and 8x levels within the collections of *D. alata* in Guadeloupe and Nigeria.

Previous study was conducted on *D. alata* of the ivorian yam gene bank by Hamon *et al.* (1992). Their investigation led to the conclusion that 82.9 % of the accessions were triploid. The present investigations lead to 61 % of tetraploid accessions and no triploid were observed. The large majority of tetraploid in a collection formerly found mainly triploid by Hamon's team suggest possible errors during determination. Such contradictory informations exist for some accessions of *D. alata* from the ivorian collection. Florido, Brazo Fuerte and Suidié were identified either tetraploid (Gamiette *et al.,* 1999) or hexaploid (Egesi *et al.,* 2002), or triploid and tetraploid (Sanou 1996). We found that Florido and Brazo had a level of 4.5x and Suidié was tetraploid. These variations show the difficulties of getting the accurate level of ploidy of *D. alata,* even by flow cytometry. Roux *et al.* (2003), discovered that in bananas, a polyploid crop like yam, the DNA content is not constant at chromosome level. The hypothesis of chromosome DNA content should be tested on yam, to explain ploidy behaviour of some accessions. In regards with what precedes, the true levels of ploidy for *D. alata* seem to be 4x, 6x and 8x. The other cases mentioned might be due experimentation to errors or chimeras. Assuming this hypothesis, the accessions collected in Côte d'Ivoire were all tetraploid. Accessions from Puerto Rico were tetraploid and hexaploid. New Caledonia is the single origin where octoploid accession is observed even if it is only one clone. Accessions from other countries of West Africa, West Indies and India can be introduced in Côte d'Ivoire with the idea to cross them with those existing in the country, as they have the same level of ploidy. For genotypes 6x from Puerto Rico and 8x from New Caledonia, their introduction will be useful for clonal selection, as their levels of ploidy differ from those from Côte d'Ivoire.

For all the studied samples, there was no complete loss of yield due to the disease. The severities noted were 1, 2 and 4. Scores 1 and 2 represented 94 % of the total, whereas score 4, indicating high sensibility, represented 6 %. This has also been showed by Sanou (1996), in Côte d'Ivoire and Mignouna *et al.* (2001), in Nigeria. The authors indicated that part of the clone Brazo fuerte, *D. alata* genotypes were more or less susceptible, depending on the strain of *Colletotrichum gleosporioides* which attacks the plant. Indeed, Brazo fuerte showed resistance to yam anthracnose throughout the years in Bouaké, as well as in Abidjan. The ratio of susceptibility of the accessions indicates that many genotypes of *D. alata* were resistant to yam anthracnose. Unfortunately, the varieties

with good level of resistance are less appreciated by consumers because of their poor cooking quality. These two parameters are particularly prefered. The positive and significant Pearson Correlation Coefficient, calculated for ploidy level and anthracnose severity, shows that the higher the ploidy level, the higher the sensibility to yam anthracnose. Tetraploid genotypes should be less susceptible to the disease than the other genotypes. Most of the accessions are tetraploid (61 %). This can explain the fact that only 4 % are very susceptible to the disease.

It is well known that *D. alata* scarcely flowers (Degras, 1986) and as many yams species, those that flower, are very often dioecious. Sixty nine percent of the accessions observed did not flower, 19 % flowered male and 12 % flowered female. No monoecious was found and there was no fruit set. Flowering is rare and female flowers tend to be more limited than male (Martin and Rhodes, 1977 ; Degras, 1986 ; Asiedu *et al.,* 1997).

CONCLUSION

All the accessions from Côte d'Ivoire were tetraploid and the majority of them did not flower. For yam breeding programmes, introduction of varieties from other countries is imperative to broaden the genetic base. It appears clearly that all male flowering clones were tetraploid, whereas female were mostly hexaploid as reported (1991). There was no fruit setting. In addition to agronomic and environmental conditions, meiotic irregularities, due to difference in ploidy levels of males and females, induce gametes with different numbers of chromosomes for both parents. That makes flowers abort because of unsuccessful fecundation. To improve the species qualities, chromosome doubling by polyhaplodisation might be a solution.

All the flowering plants were not susceptible to anthracnose. Then, breeding program to select clone combining resistance to anthracnose and other agronomic traits can be conducted with more chances of success if fruit abortion is overcome and environmental conditions are controlled.

As previously shown, susceptible varieties do not flower in our experimental conditions. Therefore, it will be difficult to create new varieties of this species, accumulating good cooking quality and resistance to anthracnose through cross pollination. Nevertheless, at IITA, Ibadan in Nigeria, profuse flowering is obtained with clones which never flowered in Côte d'Ivoire. That means the flower set is not only genetically controlled but also is influenced by environmental conditions. Therefore flowering can be induced by hormones and fruit set improved by technical approaches.

Further investigations are needed to elucidate ploidy level complexity within yam *D. alata* and establish relation with some agronomic traits.

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