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Yam (*Dioscorea rotundata* Poir.) Displays Prezygotic and Postzygotic Barriers to Prevent Autogamy in Monoecious Cultivars

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Abstract: Cultivated yam (*D. rotundata*) is a staple tuber crop in West Africa whose sexual reproduction control remains largely unknown despite its importance for plant breeding programs. In this paper, we compared self-pollination, intracultivar cross-pollination and intercultivar cross-pollination in three monoecious cultivars (Amoula, Heapala and Yassi). Results showed that pollen viability (49%) and stigma receptivity (40%) were similar in monoecious and dioecious plants, suggesting that autogamy could occur in monoecious plants. However, fruit and seed sets were significantly lower after self-pollination compared to cross-pollination. Overall, autogamy reached 11% and pollen lability was almost zero (<1%). The low percentage of pollen grains germinating on the stigma (37%) and pollen tubes reaching the ovules (25%) after self-pollination partly explained the low seed set. Strong inbreeding depression was observed after self-pollination and almost all fruits and about 75% of the seeds resulting from self-pollination showed malformations. Seed germination was also 20 times lower after self-pollination compared to cross-pollination. Sexual reproduction remained low in *D. rotundata* even after cross-pollination as fruit and seed set did not exceed 18% and 13% respectively. Moreover, comparison between intracultivar cross-pollination and self-pollination revealed intravarietal genetic diversity inside the analyzed yam cultivars. Overall, our results showed that *D. rotundata* has a very low tolerance to autogamy in monoecious cultivars and has developed pre- and postzygotic mechanisms to limit selfing.

Keywords: *Dioscorea rotundata*; allogamy; autogamy; inbreeding depression; monoecy; pollen viability; sexual reproduction; stigma receptivity

Citation: Denadi, N.; Yolou, M.; Dadonougbo, A.E.; Zoundjihékpon, J.; Dansi, A.; Gandonou, C.; Quinet, M. Yam (*Dioscorea rotundata* Poir.) Displays Prezygotic and Postzygotic Barriers to Prevent Autogamy in Monoecious Cultivars. *Agronomy* **2022**, *12*, 872. https://doi.org/10.3390/agronomy12040872

Academic Editor: Joseph M. Patt

Received: 3 March 2022
Accepted: 30 March 2022
Published: 1 April 2022

1. Introduction

Root and tuber crops play an essential role in food agriculture in many regions of the world, particularly in Sub-Saharan Africa. Main root and tuber crops cultivated in Africa are yam (*Dioscorea* spp.), cassava (*Manihot esculenta*), taro (*Colocasia esculenta*), and sweet potato (*Ipomoea batatas*). Highly prized, yam is grown in tropical regions, and West Africa is its main breadbasket with more than 95% of the world production [1]. The largest producing countries are Nigeria, Ghana, Cote d'Ivoire, Benin, Togo, and Cameroon, and they are referred to as the yam belt [2,3]. Yam cultivation constitutes an important source of food [4–6] and income [7–9] for the rural producing populations. It is an integral part of sociocultural life in West African populations [4,10]. In Benin, one of the most cultivated

species is white Guinea yam *Dioscorea rotundata* Poir. [11]. This species is also named *Dioscorea cayenensis–D. rotundata* species complex, depending on the authors [12–16]. Its cultivation is confronted with phytosanitary problems [17–23], with heavy consequences on yield [17]. Despite this, the plant benefits from little scientific research partly due to the complexity of its reproduction that limits the development of breeding programs. Recognized as a long-cycle plant, it has adopted a mainly vegetative mode of reproduction [24,25]. However, studies have shown that some cultivars of *D. rotundata* flower and produce seeds capable of a complete reproductive cycle [26–33]. The species *D. rotundata* is essentially characterized by dioecy and allogamy although monoecious plants bearing both male and female flowers on the same plant are encountered [15,24,31]; which would allow self-pollination within monoecious cultivars. However, male flowers usually flower before female flowers in *D. rotundata* [34], which can naturally limit self-fertilization. Sex identity is genetically controlled in yam with either a female heterogametic (ZZ/ZW) or a male heterogametic (XX/XY) system depending on the species [34–38]. However, spontaneous variations in sex could be observed from year to year [16].

In root and tuber crops, the reduced ability of sexual propagation is directly inherited from domestication and diversification processes and traits related to sexual reproduction are no longer highly maintained [38,39]. However, control of the plant reproductive biology is essential for efficient plant breeding [38,39]. Self-pollination is a key element in the creation of varieties in cross-pollinated plants and is of particular interest for root and tuber crops that reproduce vegetatively. Understanding the sexual reproduction of root and tuber crops is thus fundamental for the improvement of these species [40]. Autogamy and self-compatibility have been observed in monoecious root crops such as cassava (*Manihot esculenta*) and taro (*Colocasia esculenta*) [41–43]. In *D. rotundata* yams, the few studies that focused on hybridization have mentioned the problems of pollen viability, the low receptivity of female flowers and, above all, the problems of cross-incompatibility [27,28,43,44]. None of them has really addressed the issue of the possibility of autogamy within the species. While the use of improved varieties is the most effective way to increase yields and make yam production sustainable [45], it is more than necessary to move towards understanding its sexual reproduction. The aim of this paper is thus to characterize the breeding system of monoecious *D. rotundata* yam cultivars and to compare autogamy and allogamy in this species. We are first interested in whether, apart from natural dispositions (separation of the male and female flowers), *D. rotundata* has established a particular form of male or female sterility in monoecious plants. Secondly, we will analyze whether autogamy is possible in this species and whether there are pre- and/or postzygotic barriers to autogamy.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

Three yam (*D. rotundata*) cultivars (cv. Amoula, Heapala and Yassi) were used in this study. These cultivars produced monoecious, male and female plants. Tubers of 90 plants (30 monoecious, 30 male and 30 female) of each cultivar were harvested in December 2018 in a field located at Ouaké, Benin (9°39′42″ N, 1°23′5″ E). Tubers were kept on site in a bute for one month before being dug up and transferred to the attic of the 'Centre de Recherche, de Formation, d'Incubation et d'Innovation pour le Développement Agricole' (CREFIISDA, Zogbodomey, Benin, 6°56′08″ N, 1°58′24″ E) where they spent a further 3 months of dormancy. The tubers were planted at the CREFISDA site in three blocks separated by about 700 m, according to the sex of the parent plant (male, female or monoecious). In each block, cultivars were aligned on a line of 10 ridges; each repeated 3 times (fisher block design). Sowing took place on 27 April 2019 for tubers issued from monoecious and female plants and on 11 May 2019 for tubers issued from male plants in order to synchronize the flowering. The germination rate, sex ratio and flowering time of the investigated plants are reported in Table 1. The germination rate was 93.3%, 88.9%, and 90.0% for the cv. Amoula, Heapala, and Yassi, respectively. All the plants flowered. Monoecious plants produced

either male and female flowers inside the same inflorescence (mixed inflorescence) or male and female flowers on separated inflorescences (male and female inflorescences) (Figure 1A).

Table 1. Germination rate, sex ratio and flowering time of the yam (*D. rotundata*) plants used in the study.

Cultivar	Parental Sex [a]	Germination (%)	Sex Ratio (%) [a]			Flowering Time (Days after Germination) [a]		
			M	F	Mo	M	F	Mo
Amoula	M	93.3	100	0	0	71 ± 4	87 ± 5	80 ± 2
	F	86.7	15.4	84.6	0			
	Mo	100	53.3	33.3	13.3			
Heapala	M	86.7	92.3	0	7.7	68 ± 5	90 ± 4	82 ± 4
	F	83.3	0	100	0			
	Mo	96.7	34.8	20.7	44.8			
Yassi	M	96.7	96.6	0	3.4	69 ± 3	92 ± 5	80 ± 4
	F	73.3	0	86.4	13.6			
	Mo	100	60	3.3	36.7			

[a] M: male, F: female, Mo: monoecious.

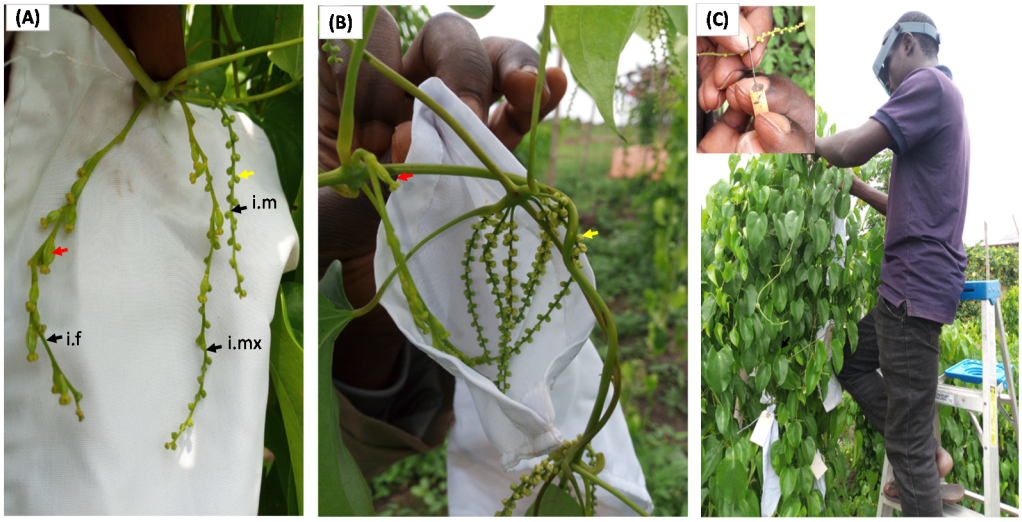

Figure 1. *Dioscorea rotundata* inflorescences and crossings methods. (**A**) Different types of inflorescences found in monoecious plants: i.f = inflorescence-bearing female flowers; i.mx = inflorescence-bearing male and female flowers; and i.m = inflorescence-bearing male flowers—the yellow and red arrows indicate male and female flowers respectively. (**B**,**C**) Different crossing methods used in this study: (**B**) inflorescence bagging for bagged intraindividual self-pollination and (**C**) pollen collection for manual pollination.

2.2. Flower Fertility Measurements

To investigate flower fertility, stigma receptivity and pollen viability were assessed on female and male flowers, respectively, of both unisexual and monoecious plants for the three cultivars. The investigated female plants were offspring of female plants, the investigated male plants were offspring of male plants, and the investigated monoecious plants were offspring of monoecious plants.

Stigma receptivity was estimated by the peroxidase activity using a colorimetric test as described by [46] on 500 flowers per cultivar (5 flowers per inflorescence and 5 inflorescences per plant for 10 monoecious and 10 female plants). After dissection under stereomicroscope, pistils were immerged for 5 min in a solution consisting of 25 mL acetate buffer, 16.5 mg $CaCl_2.2H_2O$, 12.5 mg 3-amino-9-ethylcabaole (previously dissolved in 1 mL

N-N-dimethylformamide), and 0.125 mL H_2O_2. Stigmas were observed under stereomicroscope and classified as receptive (brown coloration) or not receptive (no coloration).

Pollen viability was estimated by in vitro pollen germination test (Figure 2A) on 400 flowers per cultivar (10 flowers per inflorescence and 2 inflorescences per plant for 10 monoecious plants and 10 male plants). After dissection under stereomicroscope, the anthers were crushed between two microscope slides to release the pollen grains (often very sticky). The pollen was then transferred to microscope slides covered with a culture medium. The culture medium was composed of 4 g sucrose, 1 mg boric acid, 5 mg calcium nitrate, 0.46 g agar in 20 mL distilled water. After boiling, the medium was autoclaved and spread over the slides. The slides containing the pollen grains were then placed in sterile petri dishes containing filter paper soaked in distilled water to saturate the chamber with moisture. The whole set was placed in an oven at a temperature of 28 °C, for 24 h [15]. The slides were then observed under an optical microscope. The number of germinated pollen grains and nongerminated pollen grains were counted on five different microscopic fields per slide and the pollen viability was calculated as the ratio between the number of germinated pollen grains and the total number of pollen grains, expressed as a percentage.

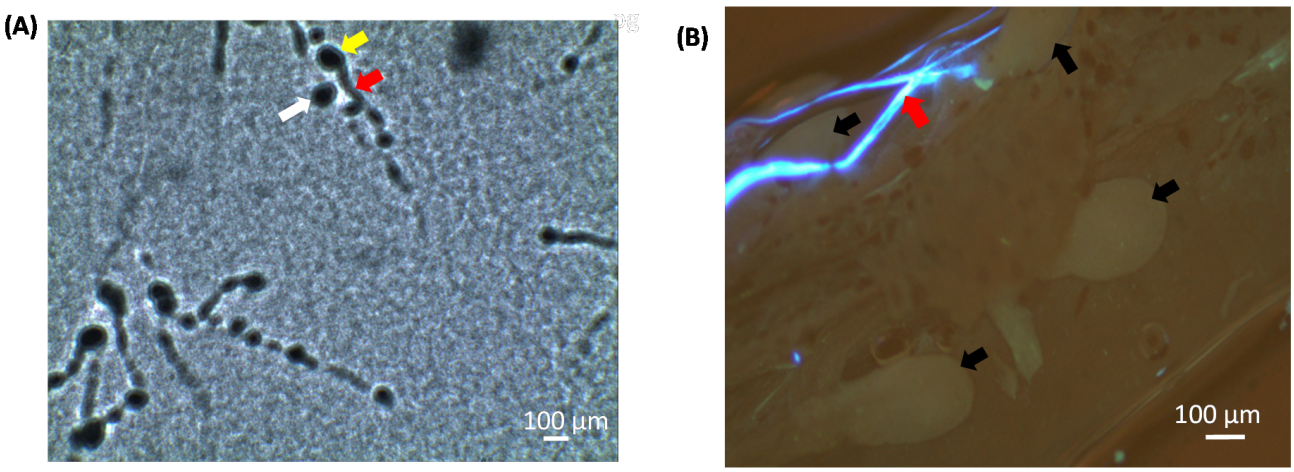

Figure 2. Pollen germination. (**A**) In vitro pollen germination for pollen viability assessment; (**B**) pollen tube growth in the style and pollen-reaching ovule. The white, yellow, red, and black arrows show, respectively, an ungerminated pollen grain, a germinated pollen grain, a pollen tube, and ovules.

2.3. Self and Cross Pollination Crossings

For each cultivar, 10 monoecious plants (offspring of monoecious plants) and one male plant (offspring of male plant) were selected for crossings. The plants were chosen according to the quantity of flowers available. Monoecious plants were used as female parent and monoecious or male plants were used as male parent depending on the crosses.

Four types of crosses were performed for each cultivar: (1) bagged intraindividual self-pollination (A_1); (2) intraindividual manual self-pollination (A_2); (3) intracultivar manual cross-pollination (C_1); and (4) intercultivar manual cross-pollination (C_2). Details of the different crosses are presented in Table 2. It has to be mentioned that strict autogamy (pollen coming from the same flower) is not possible in unisexual flowers and that what we consider in this paper as autogamy is in fact geitonogamy (pollen coming from other flowers of the same plant) [47]. For bagged intraindividual self-pollination, mixed inflorescences (bearing both male and female flowers) or close male and female inflorescences of a same plant (Figure 1A) were bagged together with a mesh to avoid insect pollination (Figure 1B). For manual pollination, a needle and magnifying glass were used to access the anthers and collect the pollen grains (Figure 1C) that were then deposited on the stigmas of the female flowers. The flowers were bagged before anthesis and after pollination with a mesh to avoid insect pollinations. The cross-pollinations were carried out during the period of

the day when the male flowers are open according to [48]. For each of the 21 cross types, a total of 110 female flowers, from an average of 10 inflorescences, were randomly selected and pollinated.

Table 2. The different types of crosses carried out.

Type of Crosses	Parents (Female × Male) [a]	Number of Pollinated Flowers [b]
bagged intraindividual self-pollination (A_1)	Amoula (Mo) × Amoula (Mo)	-
	Heapala (Mo) × Heapala (Mo)	-
	Yassi (Mo) × Yassi (Mo)	-
intraindividual manual self-pollination (A_2)	Amoula (Mo) × Amoula (Mo)	110
	Heapala (Mo) × Heapala (Mo)	110
	Yassi (Mo) × Yassi (Mo)	110
intracultivar manual cross-pollination (C_1)	Amoula (Mo) × Amoula (M)	110
	Heapala (Mo) × Heapala (M)	110
	Yassi (Mo) × Yassi (M)	110
intercultivar manual cross-pollination (C_2)	Amoula (Mo) × Heapala (M)	110
	Amoula (Mo) × Heapala (Mo)	110
	Amoula (Mo) × Yassi (M)	110
	Amoula (Mo) × Yassi (Mo)	110
	Heapala (Mo) × Amoula (M)	110
	Heapala (Mo) × Amoula (Mo)	110
	Heapala (Mo) × Yassi (M)	110
	Heapala (Mo) × Yassi (Mo)	110
	Yassi (Mo) × Amoula (M)	110
	Yassi (Mo) × Amoula (Mo)	110
	Yassi (Mo) × Heapala (M)	110
	Yassi (Mo) × Heapala (Mo)	110

[a] Mo—monoecious plant, M—male plant, [b]—no manual pollination.

2.4. Pollen Tube Growth, Fruit Set, Seed Set, and Seed Quality Assessment

For manual pollinations, 10 female flowers per cross and cultivar were harvested 48 h after pollination and fixed in FAA (ethanol 70%; formaldehyde 35%; acetic acid; 8:1:1) to investigate pollen germination on the stigma and pollen tube growth in the style (Figure 2B). The pistils were stained with 0.1% (*w*/*v*) aniline blue solution according to [49]. Flowers were dissected under stereomicroscope, the pistils were water rinsed and softened in 1N NaOH for 4 h before staining with aniline blue for 1 h. The pistils were gently squashed on a microscope slide in a drop of water and observed by means of fluorescence microscopy. Pollen grain germination was calculated as the ratio between the number of germinated pollen grains and the number of deposited pollen grains, expressed as a percentage. The fertilization rate was calculated as the ratio between the number of pollen tubes that reached the ovules and the number of pollen grains that germinated on the stigma, expressed as a percentage.

The remaining pollinated female flowers were left for fruit set and seed set assessment. The fruit set was calculated as the ratio between the number of fruits per inflorescence and the number of flowers per inflorescence (for treatment A_1) or the number of pollinated flowers per inflorescence (for treatments A_2, C_1, C_2), expressed as a percentage. A minimum of 5 inflorescences (average of 100 flowers) was followed per plant for each cultivar and cross type. The seed set was calculated as the ratio between the number of seeds per fruit and the number of ovules per flower (6 ovules per flower), expressed as a percentage. A total of 100 fruits per cross type and cultivar were analyzed for seed set assessment. As some fruit and seed malformations were observed, the proportion of misshaped fruits and seeds was also assessed for each type of cross. Fruit quality was assessed based on their three-lobed structure and seed quality was assessed based on finger palpation [15]. Moreover, seed germination was evaluated for each type of cross. Seeds were sown in

germination boxes in a mixture of compost and sand (2/3:1/3) and cultivated in a tropical greenhouse (28 °C, 90% relative humidity, 16 h/8 h photoperiod, watering 3 times a week). A total of 450 well-formed seeds were selected at a rate of 50 seeds per cultivar and type of cross. Germination was monitored for 60 days. Germination rate was calculated as the ratio between the number of germinated seeds and the number of sown seeds, expressed as a percentage [50–52].

The self-compatibility index (SCI) and self-fertility index (SFI) were determined according to Lloyd and Schoen [53]. The SCI determines the ability of an individual to produce fruits or seeds following self-fertilization. It was calculated as the ratio between the fruit set (or seed set) obtained after intra-individual manual self-pollination (A_2) and inter-cultivar manual cross-pollination (C_2). SFI is the ability of an individual to autonomously (without hand or insect intervention) produce fruits and seeds. It was calculated as the ratio between the fruit set (or seed set) obtained after bagged intraindividual self-pollination (A_1) and intraindividual manual self-pollination (A_2). SCI values above 0.2 indicate self-compatibility and SFI values above 0.2 indicate autonomous selfing [53,54].

Inbreeding depression was assessed at different developmental stages (fruit set, seed set and seed germination). It is the ratio of the relative performance of selfed (Ws) to cross-pollinated (Wc) progeny (δ = 1-(Ws/Wc) [55]. Here, selfed progeny was assessed by the intraindividual manual self-pollination (A_2) and cross-pollinated progeny was assessed by the intercultivar manual cross-pollination (C_2). Values near 0 (for a scale of 0 to 1) indicate the absence of inbreeding depression [54].

2.5. Statistical Analyses

Statistical analyses were conducted using JMP Pro.16 version 2020. The normality of the data was graphically checked (box plot and qq plot), and homoscedasticity was verified using Levene's tests. Analyses of variance with the ANOVA, Welch's test or wilcoxon test functions (according to normality and homoscedasticity) were performed to measure the effects of the cultivar and the pollination type (for the fruit and seed set, seed an embryo area, pollen tube growth, SCI, SFI, inbreeding depression) or the effects of the cultivar and the sex of the plant (for pollen viability and stigma receptivity). Post hoc comparisons between cultivars and pollination types were done using Tukey's HSD test, Welch's corrected t-test or the Wilcoxon multiple comparison t-test depending on the normality and homoscedasticity of the data. Data are shown as means ± standard error.

3. Results

3.1. Flower Fertility Was the Same in Unisexual and Monoecious D. rotundata Plants

Pollen viability and stigma receptivity were compared in monoecious and dioecious plants. Pollen viability was similar in male and monoecious plants (F = 0.0345; p = 0.8539) whatever the cultivar (F = 2.61; p = 0.0919, Figure 3A). In vitro pollen grain germination averaged 49%. Stigma receptivity was also the same in female and monoecious plants (F = 0. 2829; p = 0.5968) whatever the cultivar (F = 0.1364; p = 0.8728) and averaged 44% (Figure 3B).

3.2. Autogamy and Allogamy in Monoecious D. rotundata Plants

In order to investigate the breeding system of monoecious *D. rotundata* plants, four pollination treatments were compared: bagged intraindividual self-pollination; intraindividual manual self-pollination; intracultivar manual cross-pollination; and intercultivar manual cross-pollination. For each cultivar, results of the different crosses performed for intercultivar cross-pollination were grouped since fruit sets and seed sets were similar whatever the cultivar used as male parent (Table S1).

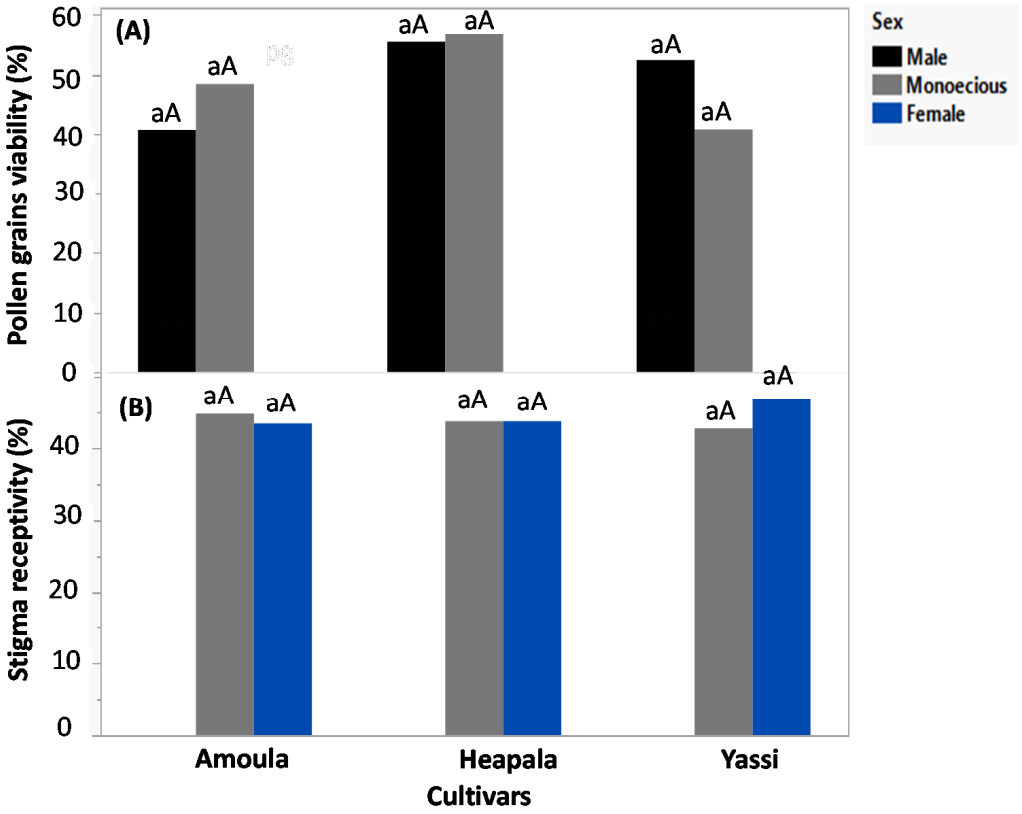

Figure 3. Pollen viability and stigma receptivity of 3 cultivars of cultivated yam (*D. rotundata*). (**A**) Pollen viability (in vitro pollen germination) in male (black) and monoecious plants (grey). (**B**) Stigma receptivity in female (blue) and mooecious (grey) plants. Values sharing the same lowercase letter among plant sexes are not significantly different at the 5% level for a same cultivar; values sharing the same capital letter among cultivars are not significantly different at the 5% level for a same sex.

The pollination treatments affected both fruit set (F = 888.73; $p < 0.0001$) and seed set (F = 718.68; $p < 0.0001$, Figure 4). Regardless of the cultivar, fruit and seed sets were higher after intercultivar (18.08% and 12.16%, respectively) and intracultivar (16.35% and 7.23%, respectively) manual cross-pollinations than after intraindividual manual (3.03% and 1.09%, respectively) and bagged (0.27% and 0.2%, respectively) self-pollinations (Figure 4). Regarding intraindividual self-pollination, manual pollination increased both the fruit set ($p < 0.0001$) and the seed set ($p < 0.0001$) compared to bagged inflorescences in the 3 cultivars. Regarding intracultivar and intercultivar cross-pollinations, differences were higher at the seed than at the fruit level (Figure 4). Fruit set was relatively higher after intercultivar than after intracultivar cross-pollinations ($p = 0.0299$) although at the cultivar level, the difference was only significant for cv. Heapala ($p = 0.0008$, Figure 4A). Seed set was also higher after intercultivar than after intracultivar cross-pollinations and the difference was significant for the 3 cultivars ($p < 0.0001$, Figure 4B). Moreover, Heapala showed a lower fruit set after both intracultivar ($p < 0.0001$) and intercultivar cross-pollinations ($p < 0.0001$) and a lower seed set after both intracultivar ($p < 0.0001$) and intercultivar ($p = 0.0023$) cross-pollinations compared to Amoula and Yassi while Yassi showed a lower seed set than Amoula ($p = 0.030$) after intracultivar cross-pollination (Figure 4).

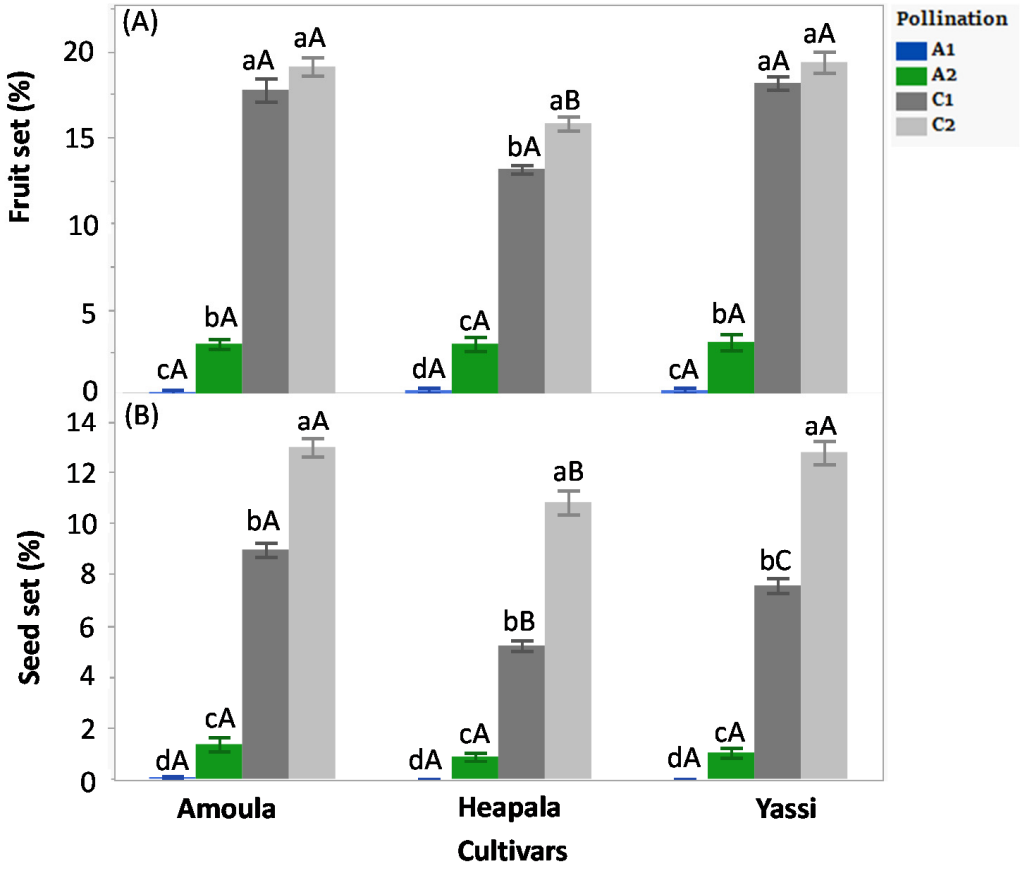

Figure 4. Fruit sets (**A**) and seed sets (**B**) after bagged intraindividual self-pollination (A_1, blue); intraindividual manual self-pollination (A_2, green); intracultivar manual cross-pollination (C_1, dark grey); and intercultivar manual cross-pollination (C_2, light grey) in monoecious yam *D. rotundata* plants. Values sharing the same lower-case letter between pollination treatments are not significantly different at the 5% level for a same cultivar; values sharing the same upper-case letter among cultivars are not significantly different at the 5% level for a same pollination treatment.

Comparison between intraindividual self-pollination and intercultivar cross-pollination showed that the self-compatibility index was below 0.2 for both fruit set (SCI_f) and seed set (SCI_s) whatever the cultivar (Table 3). In the same way, comparison between manual and bagged intraindividual self-pollinations showed that the self-fertility index was also low for both the fruit set (SFI_f) and seed set ($SFI_{s)}$ in the three cultivars (Table 3). Overall, autogamy reached 11% and pollen lability was almost zero (<1%). We observed a high inbreeding depression when comparing manual self-pollination and intercultivar cross-pollination for both fruit set ($\delta > 0.8$) and seed set ($\delta > 0.9$) whatever the cultivar (Table 3).

Table 3. Self-compatibility (SCI), self-fertility (SFI) and inbreeding depression (δ) index at fruit and seed set levels in cultivated yam *D. rotundata*.

		Amoula	Heapala	Yassi	ANOVA (A) and Wilcoxon (W) Tests	
SCI [a]	Fruit Set (SCI_f)	0.16 ± 0.02a	0.19 ± 0.03a	0.16 ± 0.03a	A	$F = 0.97$; $p = 0.38$
	Seed Set (SCI_s)	0.10 ± 0.02a	0.08 ± 0.02a	0.09 ± 0.02a	A	$F = 0.14$; $p = 0.86$
SFI [a]	Fruit Set (SFI_f)	0.01 ± 0.01a	0.02 ± 0.01a	0.02 ± 0.01a	A	$F = 0.12$; $p = 0.88$
	Seed Set (SFI_s)	0.00 ± 0.00a	0.000 ± 0.00a	0.00 ± 0.00a	W	$\chi^2 = 6.42$; $p = 0.08$
Inbreeding depression [a]	Fruit set	0.84 ± 0.02a	0.81 ± 0.03a	0.84 ± 0.03a	W	$F = 0.61$; $p = 0.54$
	Seed set	0.90 ± 0.02a	0.92 ± 0.02a	0.91 ± 0.02a	A	$F = 0.33$; $p = 0.71$
	Seed germination	0.96 ± 0.01a	0.96 ± 0.02a	0.95 ± 0.01a	A	$F = 0.49$; $p = 0.61$

[a] Values sharing the same lower-case letters between cultivars are not significantly different at the 5% level.

Fruit and seed set results could be partly explained by the pollen germination on stigma and the pollen tube growth in the style. Pollen tube growth analyses showed that the pollination treatment affected both the germination of pollen grains at the stigma surface (F = 37.45; $p < 0.001$) and the percentage of pollen tubes that reached the ovules ($\chi^2 = p < 0.0001$) (Figure 5). Pollen germination was lower after manual self-pollination (37%) than after cross-pollination (76%) ($p < 0.0001$) but it was similar for intra- and intercultivar cross-pollinations in the three cultivars (Figure 5A). No differences were observed for the pollen germination percentage among the three cultivars (F = 0.02; $p = 0.97$, Figure 5A). Only 25% of the pollen tubes that germinated reached the ovules after self-pollination while about 75% of the pollen tubes reached the ovules after intra- and intercultivar cross-pollinations (Figure 5B). However, the percentage of pollen tubes reaching the ovules was significantly higher after intercultivar than after intracultivar cross-pollinations ($p < 0.001$) whatever the cultivar (Figure 5B). As previously observed for seed set, Heapala showed a lower percentage of pollen tubes reaching the ovules after intracultivar ($p = 0.0006$) and intercultivar ($p = 0.0017$) cross-pollinations compared to Amoula and Yassi, and Yassi showed a lower percentage of pollen tubes reaching the ovules than Amoula ($p = 0.030$) after intracultivar cross-pollination (Figure 5B).

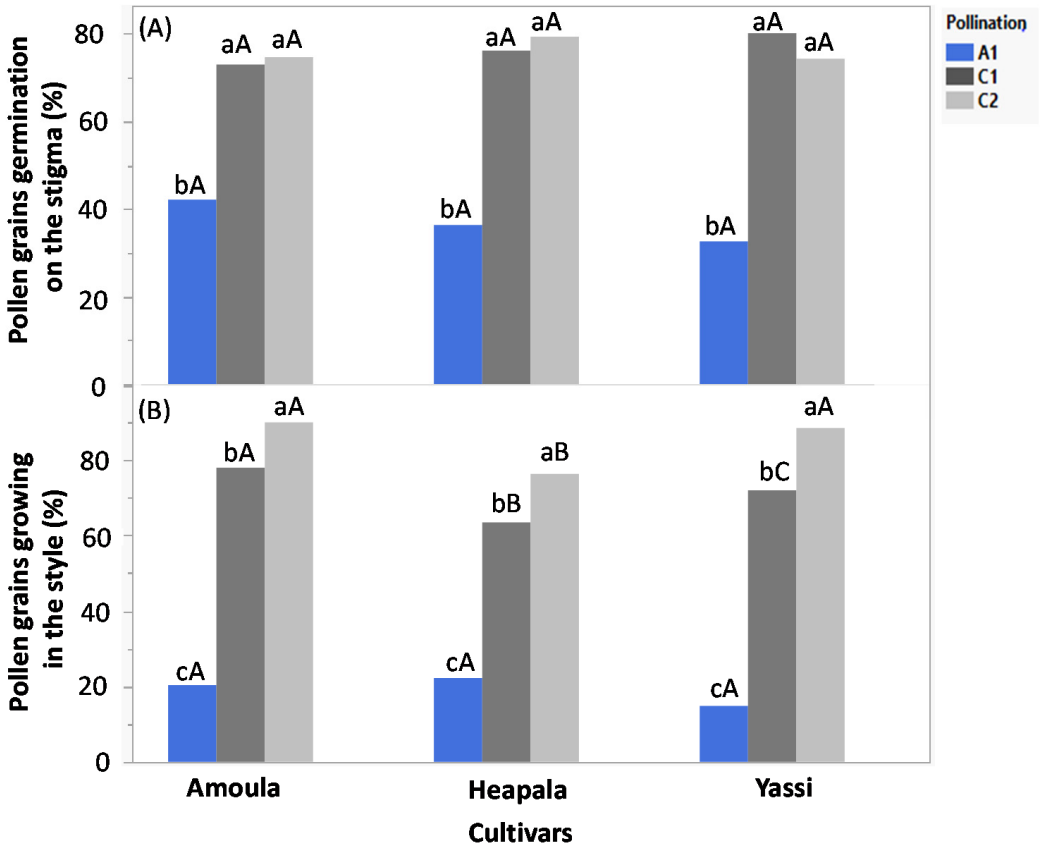

Figure 5. Pollen germination on stigma and pollen tube growth after intraindividual manual self-pollination (A_2, blue), intracultivar manual cross-pollination (C_1, dark grey) and intercultivar manual cross-pollination (C_2, light grey) in monoecious yam *D. rotundata* plants. (**A**) Percentage of pollen grains on the stigma that initiated a pollen tube. (**B**) Percentage of germinating pollen grains which pollen tube reached the ovules. Values sharing the same lower-case letter between pollination treatments are not significantly different at the 5% level for a same cultivar; values sharing the same upper-case letter among cultivars are not significantly different at the 5% level for a same pollination treatment.

Fruit set and seed set evaluation were based on the total number of fruits and seeds produced. However, misshapen fruits and seeds were also observed (Figure 6). Almost

all fruits and about 75% of the seeds resulting from self-pollination (A_1 and A_2) were misshaped while 66% of the fruits and 50% of the seeds from intracultivar cross-pollination (C_1) and 50% of the fruits and 37% of the seeds from intercultivar cross-pollination (C_2) were misshaped (Table S2). Well-shaped fruits have 3 lobes (Figure 6A) and well-shaped seeds have large wings with an ovate and smooth embryo (Figure 6D). Two types of malformations were observed within the fruits and the seeds. The first type concerned fruits having only one or two lobes (Figure 6B) and seeds with a rough embryo (Figure 6E). The second type concerned globular fruits without lobes (Figure 6C) and often without seeds or with seeds with small wings and small round and dark embryo (Figure 6F). Misshapen fruits and seeds of the first type were mainly observed after cross-pollination and misshapen fruits and seeds of the second type were mainly observed after self-pollination. Pollination treatment affected both seed ($F = 405.61$; $p < 0.0001$, Figure 7A) and embryo ($F = 55.7$; $p < 0.0001$, Figure 7B) area. Seeds and embryos resulting from self-pollination were smaller than the ones resulting from intracultivar and intercultivar cross-pollinations ($p < 0.0001$). However, the size of seeds ($p = 0.058$) and embryos ($p = 0.46$) were similar between intracultivar and intercultivar cross-pollinations. Furthermore, Yassi produced smaller seeds than Amoula and Heapala after intra- ($p = 0.0141$) and intercultivar ($p < 0.0001$) cross-pollinations (Figure 7A) and smaller embryos than the other cultivars after self-pollination ($p = 0.0011$) and intracultivar cross-pollination ($p = 0.0218$, Figure 7B). Regarding seed germination (Figure 7C), most of the seeds resulting from self-pollination did not germinate (<4%) compared to intracultivar (62.96%) and intercultivar cross-pollinations (85.16%) ($F = 5659.10$; $p < 0.0001$). Strong inbreeding depression ($\delta = 0.96$) was also observed for germination rate (Table 3). The germination percentage was also higher after intercultivar than after intracultivar cross-pollinations ($p < 0.0001$), whatever the cultivar. Although the seed germination percentage was similar among cultivars for self-pollination and intercultivar cross-pollination, Heapala had a significantly lower seed germination rate compared to Amoula and Yassi ($p < 0.0001$, Figure 7C) after intracultivar cross-pollination.

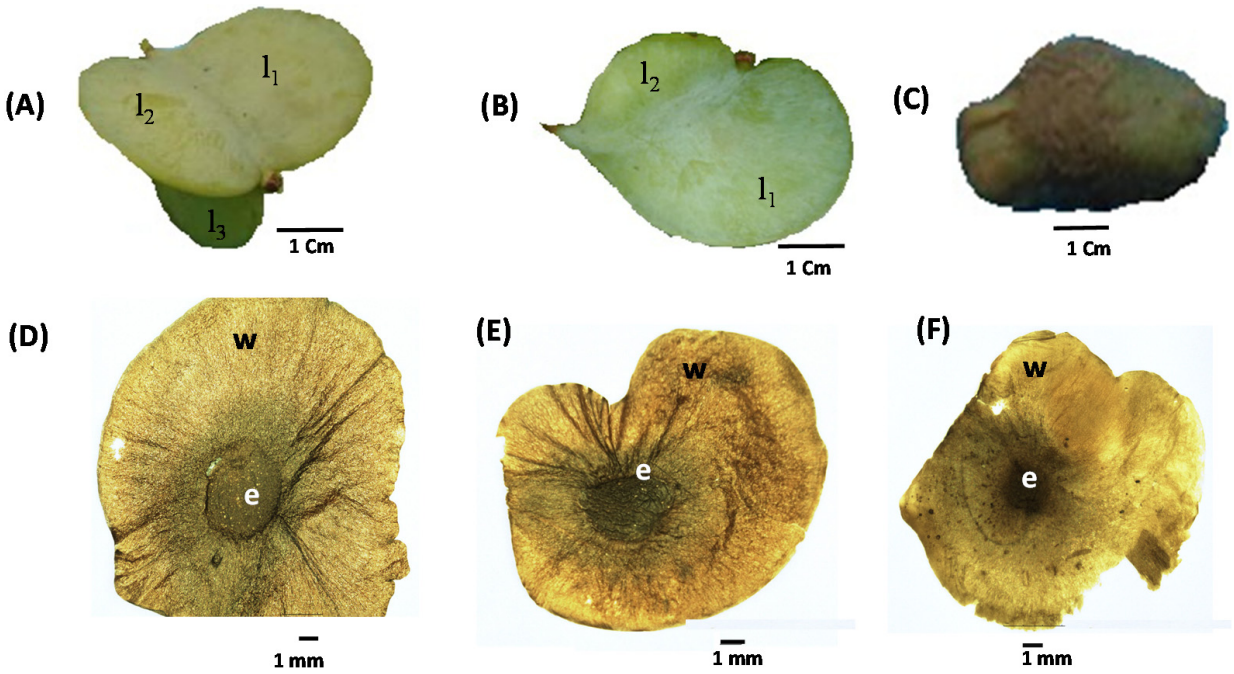

Figure 6. Structure of well-shaped (**A**,**D**) and misshapen (**B**,**C**,**E**,**F**) fruits (**A**–**C**) and seeds (**D**–**F**). w = wing; e = embryo; l = lobe.

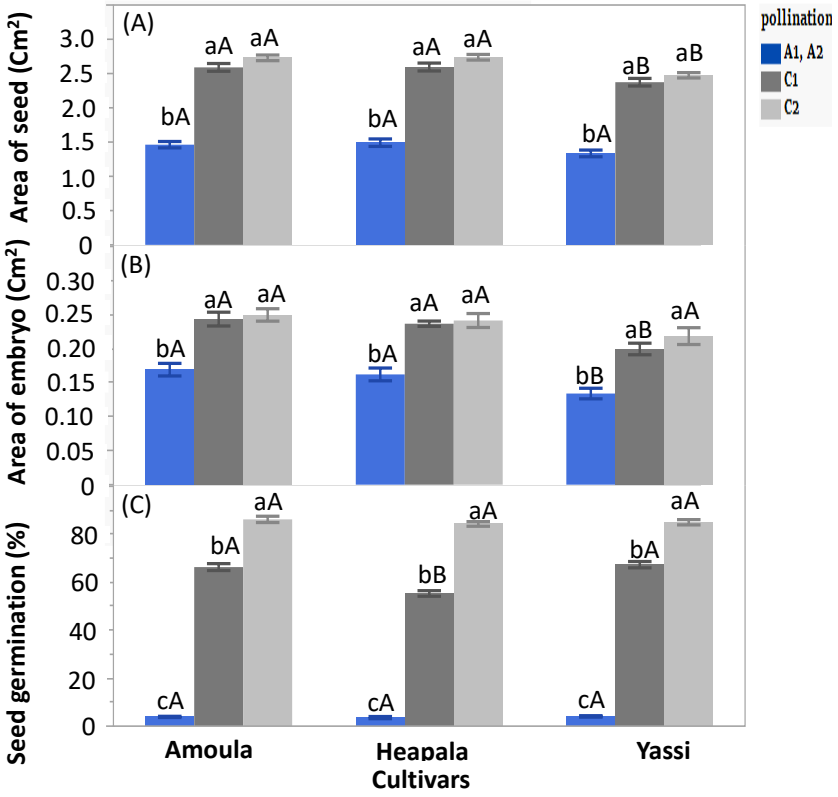

Figure 7. Quality of seeds after intraindividual self-pollination (A_1 + A_2, blue), intracultivar manual cross-pollination (C_1, dark grey) and inter-cultivar manual cross-pollination (C_2, light grey) in monoecious yam *D. rotundata* plants. (**A**) Seed area, (**B**) embryo area, (**C**) seed germination. Values sharing the same lower-case letter between pollination treatments are not significantly different at the 5% level for the same cultivar; values sharing the same upper-case letter among cultivars are not significantly different at the 5% level for the same pollination treatment.

4. Discussion

Sex determination is not stable in *D. rotundata* and although it is mainly characterized by dioecy, monoecious individuals are encountered [15,24,31]. In this paper, we focused on the reproduction of monoecious plants, investigated their fertility and analyzed their breeding system. Our results showed that male and female flowers of monoecious individuals were as fertile as the flowers of male and female individuals. Low pollen viability was often reported as a limitation to sexual reproduction in yam [43]. In addition to pollen viability, the timing and limited duration of opening of male flowers during the day is another factor that may limit the pollination process of *D. rotundata* yams [15,48]. In our study, pollen viability was assessed by in vitro pollen germination and averaged 49%. This value was similar to the pollen viability observed by Zoundjihékpon [15] using the same method; she showed that the pollen germination rate averaged 45% in West African *D. rotundata* cultivars collected in Côte d'Ivoire [15]. Other studies showed very wide ranges for yam pollen viability among cultivars. For example, pollen viability ranged from 0.3% to 85% in *D. cayenensis* from Nigeria [44], from 9% to 62% for cultivars of *D. rotundata* from Côte d'Ivoire [56] or from 20% to 98% in *D. alata* [57]. In contrast to these studies, pollen viability was quite stable among cultivars in our study. Female fertility was less investigated than pollen viability in yam. Stigma receptivity averaged 40% in our study whatever the cultivar. Previous works also reported low stigma-receptivity rates within *D. rotundata* cultivars [29,43]. Our results showed that there was no particular male or female sterility problem in monoecious individuals compared to male or female individuals in the analyzed cultivars. This contrasts with the observations of Akoroda [44] who rather observed that pollen from monoecious individuals had a lower viability compared to that

of dioecious individuals. Although the pollen viability and the stigma receptivity remained low, our results suggested that monoecious yam plants could be used both as male and female parents in crosses.

Despite the flower fertility of monoecious plants, the observed fruit and seed sets were lower after self-pollination than after cross-pollination and self-compatibility and self-fertility index were below the threshold of 0.2 [53] in our study, suggesting that the cultivated yam *D. rotundata* had almost no self-fertilization capacity (<1%). However, a low tolerance to autogamy of 18% for fruit set and 11% for seed set was observed, showing that autogamy was not completely prevented. Autogamy has been observed in monoecious cross-pollinated plants such as cassava and taro [41–43]. The low rate of self-compatibility observed in *D. rotundata* suggests that there are prezygotic and/or postzygotic barriers directed against autogamy. Plants have evolved a number of devices to limit autogamy [47]. In yam, autogamy is naturally limited by the development of unisexual flowers and the delay between the male and female flowering [43]. In addition, our results revealed a lower pollen germination on the stigma and a lower pollen tube growth in the style after self-pollination compared to cross-pollination. Indeed, more than 75% of the pollen tubes failed to reach and penetrate the ovule after self-pollination which may explain the low seed set. This observation shows that yam develops prezygotic barriers to autogamy. Nongermination of pollen grains on the stigma and cessation of pollen tube growth in the style are observed in self-incompatible plants [58]. Self-incompatibility has been reported in more than 100 plant families and occurs in approximately 40% of species [59]. Genetic self-incompatibility is a well-described mechanism preventing self-pollination and, in most angiosperms, it is controlled by a single multiallelic locus, termed S-locus, though systems controlled by two (or more) loci have also been described in certain species [60]. The genetic control of prezygotic barriers to autogamy are not known in yam to the best of our knowledge. With the release of yam draft genome sequence, several studies were developed to better understand the genetic control of sexual reproduction in yam [35,38,61,62]. Genome-wide association approach in *D. alata* identified major genetic barriers to reproduction in yam on chromosomes 1 and 6 [38] and genomic regions linked to sex and cross-compatibility on chromosomes 1, 6 and 17 [62]. Moreover, Super-SAGE transcriptome profiling in *D. rotundata* identified 88 tags differently expressed in male, female and monoecious yam plants [61]. To the best of our knowledge, no genetic sequence similar to the S-locus has been described in yam up to now. Despite prezygotic barriers, we observed that some pollen grains succeeded to germinate on stigmas of the same plant and resulting pollen tubes reached the ovules. The probability of these two events occurring simultaneously was about 9.25%, a value close to the degree of autogamy (11%) observed within cultivars in terms of seed production. This suggests that there are pathways to bypass the prezygotic mechanisms that prevent autogamy in *D. rotundata*. Plant species have evolved autogamous self-pollination as a means of reproductive assurance under pollination-uncertain environments [47]. Such reproductive assurance seems to have evolved across all sexual and breeding systems including monoecy, dioecy, and self-incompatibility [47].

In addition to prezygotic barriers, we also observed postzygotic barriers to autogamy in monoecious *D. rotundata* cultivars. Indeed, most of the fruits and the seeds resulting from self-pollination were misshaped with a high percentage of seedless fruits. These results suggested that there were problems during seed development. Moreover, seeds resulting from self-pollination had a very low germination percentage. Our results also showed a strong inbreeding depression in relation to fruit and seed set and seed germination (δ = 0.81, 0.87 and 0.95, respectively). Autogamy and geitonogamy lead to homozygosity and could thus lead to inbreeding depression because of the accumulation of lethal alleles [58]. Autogamy is observed in monoecious and dioecious species and often resulted in inbreeding depression [47]. Similar to yam, hemp (*Cannabis sativa*) is a dioecious species but there are also monoecious cultivars [63,64]. The control of self-pollination in monoecious hemp cultivars has allowed the development of self-pollinating lines that are valuable from an agronomical point of view [63,64]. Monoecious cultivars

facilitate the harvest of both stems and seeds by reducing crop heterogeneity [65]. However, self-pollinated progeny may show inbreeding depression [66], resulting from increased expression of homozygous recessive traits. Self-pollinated progeny are often smaller with lower yields than cross-pollinated progeny [67]. Similarly, most poplar species are dioecious but andromonoecious and gynomonoecious individuals that showed tolerance to self-fertilization were observed in some poplar species (*Populus. alba, P. davidiana, P. simonii et P. deltoids*) [68]. However, a self-fertilized poplar clone may have little chance to have progeny and survive because of inbreeding depression [69]. In their study on *Bidens sandvicensis*, Schultz et al. [69] found a high inbreeding depression ($\delta = 0.94$) in hermaphrodite flowers of this gynodioecious species despite moderate levels of self-fertilization (25–50%). They hypothesized that selective interference might maintain the high level of inbreeding depression found in this species [69]. For its part, *Sagittaria latifolia* exhibited both monoecious and dioecious breeding systems and selfing rates were observed in both systems but marker-based estimates of inbreeding depression ($\delta = 0.83$–0.84) indicated strong selection against inbred offspring in both monoecious and dioecious populations [70]. However, it has to be mentioned that self-pollination did not always lead to inbreeding depression and that repeated selfing results in purging of deleterious recessive alleles, thereby avoiding inbreeding depression [47].

Our results also showed that even after intercultivar cross-pollination, fruit and seed set did not exceed 18% and 13% respectively and that 50% of the fruits and 37% of the seeds were misshaped. This suggests that sexual reproduction remained low in *D. rotundata* even after cross-pollination. Mondo et al. [71] reported also pre- and postzygotic barriers in intra- and interspecific crosses in *Dioscorea* spp. The reduced ability of sexual reproduction in root and tuber crops could be directly inherited from domestication and diversification processes in which traits related to sexual reproduction were no longer maintained or even counter-selected due to the associated costs [38,39]. Shivana [47] reported that a major issue during yam hybridization activities is the low cross-compatibility rates among cultivars (~23 and 31% for *D. rotundata* and *D. alata*, respectively). Indeed, Zoundjihékpon [15] showed very high rates of fruit and seed malformation in *D. rotundata*, ranging from 0 to 91.83% and even reaching 100% in some cultivars. In the study of Sadik and Okereke [28], fruit and seed abortions ranged from 38 to 86%. In *D. alata*, manual cross-pollinations were performed using 33 parental combinations [72] and the fruit sets ranged from 0% to 35% with an average of 24% while the seed sets ranged from 0 to 33% with an average of 30%. We did not observe such variation among intercultivar crosses in our study. However, in our study, cross-pollinated seeds showed a size similar to that described by Assaba et al. [52] and a germination rate of 73%, close to the 68% observed by Yolou [32] in Benin. Furthermore, the results we observed for fruit set, seed set, pollen tube growth, seed size, and seed germination after intracultivar cross-pollination were closer to the results obtained for intercultivar cross-pollination than for self-pollination, suggesting an intracultivar diversity within *D. rotundata*. Indeed, several studies showed high intracultivar genetic diversity within *D. rotundata* cultivars [73–78]. Yolou [32] confirmed intracultivar diversity in accessions grown in Benin, including the cultivars used in this study. Thus, the idea that each cultivar is composed of genetically related clones [77] should be rethought in yam and plant-breeding programs are required to produce homogeneous varieties.

The breeding process of *Dioscorea* spp. is very long and lasts about 8–10 years because of the low multiplication rate of propagules and the existence of a juvenile phase during the seminal generation [71]. Indeed, seed germination is low, and plants issued from seeds show a low flowering rate and need to be propagated clonally before a reasonable assessment of their characteristics can be done. A long breeding process is also observed in other tuber and root crops such as cassava or sweet potato [79–81]. In cassava, the need to accelerate flowering and improve reproductive cycles has been raised to facilitate breeding programs [82–87]. The selection of parental plants with good characteristics (i.e., good tuber quality, pest and virus resistance etc.) is thus essential in breeding programs. Our study revealed that monoecious plants could serve as both male and female parents in

D. rotundata, allowing the identification of interesting flowering monoecious genotypes that could be used as both male and female parents in crossings. Moreover, the use of monoecious plants could compensate the scarcity of female plants observed in cultivated yam [27,88,89].

5. Conclusions

The results of this study showed that, although flowers of monoecious plants are as fertile as flowers of dioecious plants, cultivated yam *D. rotundata* has a very low tolerance of autogamy in monoecious cultivars. In addition to separation of male and female flowers and nonsynchronization of male and female flowering, yam has developed prezygotic and postzygotic mechanisms to limit autogamy. This is one of the factors limiting the genetic improvement of the species apart from the low flowering and fruiting rates. More studies are required to gain a deeper understanding of the reproductive system of this species and of its genetic control, in order to effectively circumvent the obstacles to self-pollination and increase the success of breeding programs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12040872/s1, Table S1: Fruit set and seed set for the different crosses performed; Table S2: Percentage of misshaped fruits and seeds for the different crosses performed.

Author Contributions: Conceptualization, N.D., C.G., J.Z., A.D. and M.Q.; methodology, N.D. and M.Q.; formal analysis, N.D.; investigation, N.D., M.Y. and A.E.D.; resources, N.D. and A.D.; writing—original draft preparation, N.D. and M.Q.; writing—review and editing, N.D., M.Y., A.E.D., J.Z., A.D., C.G and M.Q.; visualization, N.D.; supervision, J.Z., A.D., C.G. and M.Q.; project administration, C.G. and M.Q.; funding acquisition, M.Q. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful to the 'Centre de Recherche, de Formation, d'Incubation et d'Innovation pour le Développement Agricole' (CREFIISDA, Benin) for access to their site. We are also grateful to Agban Gabin, Assaba Elie, Sonon-Biti Jacques and Ani Leocadie for their technical assistance. N.D. is grateful to the International Action Committee (CAI) of UCLouvain for the funding of his PhD grant. The authors thank the Fondation Universitaire de Belgique for its contribution to publication fees.

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

References

1. FAO. FAOSTAT. Available online: http://www.fao.org/faostat/en/data/QL (accessed on 15 October 2021).
2. Coursey, D.G. *Yams. An Account of the Nature, Origins, Cultivation and Utilization of the Useful Members of the Dioscoreaceae*; Tropical Agriculture Series; Longmans: London, UK, 1967.
3. Scarcelli, N.; Cubry, P.; Akakpo, R.; Thuillet, A.-C.; Obidiegwu, J.E.; Baco, M.N.; Otoo, E.; Sonké, B.; Dansi, A.; Djedatin, G.; et al. Yam genomics supports West Africa as a major cradle of crop domestication. *Sci. Adv.* **2011**, *5*, 1947. [CrossRef] [PubMed]
4. Zannou, A.; Ahanchédé, A.; Struik, P.C.; Richards, P.; Zoundjihékpon, J.; Tossou, R.; Vodouhè, S. Yam and cowpea diversity management by farmers in the Guinea-Sudan transition zone of Benin, NJAS-Wageningen. *J. Life Sci.* **2004**, *52*, 393–420.
5. Obidiegwu, J.E.; Akpabio, E.M. The geography of yam cultivation in southern Nigeria: Exploring its social meanings and cultural functions. *J. Ethnic Foods* **2017**, *4*, 28–35. [CrossRef]
6. Omohimi, C.I.; Piccirillo, C.; Roriz, M.; Ferraro, V.; Vasconcelos, M.; Sanni, L.O.; Tomlins, K.; Pintado, M.M.; Abayomi, L.A. Study of the proximate and mineral composition of different Nigerian yam chips, flakes and flours. *J. Food Sci. Technol.* **2017**, *55*, 42–51. [CrossRef] [PubMed]
7. Sonibare, M.A.; Asiedu, R.; Albach, D.C. Genetic diversity of *Dioscorea dumetorum* (Kunth) Pax using amplified fragment length polymorphisms (AFLP) and cpDNA. *Biochem. Syst. Ecol.* **2010**, *38*, 320–334. [CrossRef]

8. Loko, Y.L.; Dansi, A.; Linsoussi, C.; Assogba, P.; Dansi, M.; Vodouhè, R.; Akoegninou, A.; Sanni, A. Current status and spatial analysis of Guinea yam (*Dioscorea cayenensis* Lam.-D. *rotundata* Poir. complex) diversity in Benin. *Int. Res. J. Agric. Sci. Soil Sci.* **2013**, *3*, 219–238.
9. Mignouna, D.B.; Akinola, A.A.; Abdoulaye, T.; Alene, A.D.; Manyong, V.; Maroya, N.G.; Aighewi, B.A.; Kumar, L.P.; Balogun, M.; Lopez-Montes, A.; et al. Potential returns to yam research investment in sub-Saharan Africa and beyond. *Outlook Agric.* **2020**, *49*, 215–224. [CrossRef]
10. Norman, M.J.T.; Pearson, C.J.; Searle, P.G.E. *Tropical Food Crops in Their Environment*; Cambridge University Press: Cambridge UK, 1995; pp. 305–316.
11. Dansi, A.; Mignouna, H.D.; Zoundjihékpon, J.; Sangare, A.; Ahoussou, N.; Asiedu, R. Identification of some Benin Republic's Guinea yam (*Dioscorea cayenensis/Dioscorea rotundata* complex) cultivars using randomly amplified polymorphic DNA. *Genet. Resour. Crop Evol.* **2000**, *47*, 619–625. [CrossRef]
12. Miège, J. Notes sur les especes Dioscorea cayenensis Lamk. et Dioscorea rotundata Poir. In *Yams (Ignames)*; Miège, J., Lyonga, S.N., Eds.; Oxford University Press, Oxford, UK, 1982; pp. 367–375.
13. Hamon, P.; Hamon, S.; et Toure, B. Les ignames cultivées du complexe *Dioscorea cayenesis-rotundata* de Côte d'Ivoire. In *Inventaire et Description des Cultivars Traditionnels*; Faculté des Sciences et Techniques et l'International Board for Plant Genetic Resources: Rome, Italy, 1986.
14. Hamon, P. Structure, Origine Génétique des Ignames Cultivées du Complexe *Dioscorea cayenensis-rotundata* et Domestication des Ignames en Afrique de l'Ouest. Ph.D. Thesis, Université Paris XI, Paris, France, 1987.
15. Zoundjihékpon, J. Biologie de la Reproduction et Génétique des Ignames Cultivées de L'Afrique de L'ouest, *Dioscorea cayenensis-rotundata*. Ph.D. Thesis, Université Nationale de Côte d'Ivoire, Abidjan, Côte d'Ivoire, 1993.
16. Dansi, A.; Mignouna, H.D.; Zoundjihékpon, J.; Sangare, A.; Asiedu, R.; Quin, F.M. Morphological diversity, cultivar groups and possible descent in the cultivated yams (*Dioscorea cayenensis/D. rotundata*) complex in Benin Republic. *Genet. Resour. Crop Evol.* **1999**, *46*, 371–388. [CrossRef]
17. Bousalem, M.; Dallot, S.; Guyader, S. The use of phylogenetic data to develop molecular tools for the detection and genotyping of Yam mosaic virus. Potential application in molecular epidemiology. *J. Virol. Methods* **2000**, *90*, 25–36. [CrossRef]
18. Amusa, N.A.; Adegbita, A.A.; Muhammed, S.; Daiyewu, R. Yam diseases and its management in Nigeria. *Afr. J. Biotechnol.* **2003**, *2*, 497–502. [CrossRef]
19. Coyne, L.D.; Tchabi, A.; Baimey, H.; Labuschagnec, N.; Rotifa, I. Distribution and prevalence of nematodes (Scutellonema bradys and Meloidogyne spp.) on marketed yam (*Dioscorea* spp.) in West Africa. *Field Crops Res.* **2006**, *96*, 142–150. [CrossRef]
20. Egesi, C.N.; Odu, B.O.; Ogunyemi, S.; Asiedu, R.; Hughes, J. Evaluation of water yam (*Dioscorea alata* L.) germplasm for reaction to yam anthracnose and virus diseases and their effect on yield. *J. Phytol.* **2007**, *155*, 536–543. [CrossRef]
21. Ogaraku, A.O.; Usman, H.O. Storage Rot of Some Yams (*Dioscorea* spp.) In Keffi and Environs, Nasarawa State, Nigeria. *Patnsuk J.* **2008**, *2*, 22–27.
22. Assala, S.; Alegbejo, M.D.; Kashina, B.; Banwo, O.O.; Asiedu, R.; Lava-Kumar, P. Distribution and incidence of viruses infecting yam (*Dioscorea* spp.) in Nigeria. *Glob. J. Biosci. Biotechnol.* **2012**, *1*, 163–167.
23. Ayisah, K.D.; Gumedzoe, Y.M.D. Genetic diversity among yam mosaic virus (YMV) isolates infecting yam of the complex *Dioscorea cayenensis rotundata* in Togo. *Int. J. Biol. Chem. Sci.* **2012**, *6*, 1090–1101. [CrossRef]
24. Akoroda, M.O. Floral biology in relation to hand pollination of white yam. *Euphytica* **1984**, *2*, 831–838. [CrossRef]
25. Dumont, R.; Dansi, A.; Vernier, P.; Zoundjihékpon, J. Pratiques traditionnelles conduisant à *Dioscorea rotundata*. In *Biodiversité et Domestication des Ignames en Afrique de L'ouest*; Collection Repère; CIRAD: Montpelier, France, 2005.
26. Miège, J. Contribution à L'étude Systématique de Dioscorea Ouest Africaines. Ph.D. Thesis, University of Paris: Paris, France, 1952.
27. Sadik, S.; Okereke, O.U. Flowering, Pollen Grain Germination, Fruiting, Seed Germination and Seedling Development of White Yam, *Dioscorea rotundata* Poir. *Ann. Bot.* **1975**, *39*, 597–604. [CrossRef]
28. Sadik, S.; Okereke, O.U. A new approach to improvement of yam *Dioscorea rotundata*. *Nat. Cell Biol.* **1975**, *254*, 134–135. [CrossRef]
29. Zoundjihékpon, J. Floral biology of cultivated yams of West Africa, *Dioscorea cayenensis-rotundata*. In *Plant Sciences Today*; de Kouchkovsky, Y., Ed.; Les Colloques: Paris, France, 1991; Volume 59, p. 84.
30. Dumont, R.; Zoundjihékpon, J.; Vernier, P. Origine et diversité des ignames *Dioscorea rotundata* Poir. Comment le savoir-faire des paysans africains leur permet d'utiliser la biodiversité sauvage dans l'agriculture. *Cah. Agric.* **2010**, *19*, 255–261.
31. Yolou, M.; Anizehou, I.; Dossou-Yovo, R.; Akoegninnou, A.; Zongo, J.D.; Zoundjihekpon, J. Etat des lieux de la reproduction sexuée des ignames africaines *Dioscorea cayenensis–Dioscorea rotundata* cultivées au Bénin. *Int. J. Biol. Chem. Sci.* **2015**, *9*, 737–750. [CrossRef]
32. Yolou, M. Genetic Diversity of Cultivated African Yams (*Dioscorea cayenensis—D. rotundata* Complex and *D. dumetorum*) and the Perception of Peasants in Central Benin about Intellectual Poperty Rights. Ph.D. Thesis, University of Abomey-Calavi, Abomey-Calavi, Benin, 2016.
33. Zoundjihekpon, J.; Hamon, P.; Hamon, S.; Tio-Toure, B. Relations entre germination, mise à fleurs et niveau de prélèvement des semenceaux d'ignames du complex *Dioscorea cayenensis–rotundata*. *Agron. Afr.* **1995**, *7*, 223–235.
34. Martin, F.W. Sex Ratio and Sex Determination in *Dioscorea*. *J. Hered.* **1966**, *57*, 95–99. [CrossRef]

35. Tamiru, M.; Natsume, S.; Takagi, H.; White, B.; Yaegashi, H.; Shimizu, M.; Terauchi, R. Genome sequencing of the staple food crop white Guinea yam enables the development of a molecular marker for sex determination. *BMC Biol.* **2017**, *15*, 86. [CrossRef] [PubMed]
36. Cormier, F.; Lawac, F.; Maledon, E.; Gravillon, M.-C.; Nudol, E.; Mournet, P.; Vignes, H.; Chaïr, H.; Arnau, G. A reference high-density genetic map of greater yam (*Dioscorea alata* L.). *Theor. Appl. Genet.* **2019**, *132*, 1733–1744. [CrossRef]
37. Denadi, N.; Gandonou, C.; Missihoun, A.A.; Zoundjihékpon, J.; Quinet, M. Plant Sex Prediction Using Genetic Markers in Cultivated Yams (*Dioscorea rotundata* Poir.) in Benin. *Agronomy* **2020**, *10*, 1521. [CrossRef]
38. Cormier, F.; Martin, G.; Vignes, H.; Lachman, L.; Cornet, D.; Faure, Y.; Arnau, G. Genetic control of flowering in greater yam (*Dioscorea alata* L.). *BMC Plant Biol.* **2021**, *21*, 163. [CrossRef]
39. Meyer, R.S.; Purugganan, M.D. Evolution of crop species: Genetics of domestication and diversification. *Nat. Rev. Genet.* **2013**, *14*, 840–852. [CrossRef]
40. Sedgley, M.; Griffin, A. *Sexual Reproduction of Tree Crops*; Academic Press: New York, NY, USA, 1989.
41. Alves, A.A.C. Cassava botany and physiology. In *Cassava: Biology, Production and Utilization*; Hil-locks, R.J., Thresh, J.M., Bellotti, A.C., Eds.; CABI Publishing: New York, NY, USA, 2002; pp. 67–89.
42. Ivancic, A.; Lebot, V. *The Genetics and Breeding of Taro*; CIRAD: Montpellier, France, 2000.
43. Lebot, V.; Abraham, K.; Kaoh, J.; Rogers, C.; Molisalé, T. Development of anthracnose resistant hybrids of the Greater Yam (*Dioscorea alata* L.) and interspecific hybrids with *D. nummularia* Lam. *Genet. Resour. Crop Evol.* **2019**, *66*, 871–883. [CrossRef]
44. Akoroda, M.O. Pollination management for controlled hybridization of white yam. *Sci. Hortic.* **1985**, *25*, 201–209. [CrossRef]
45. Ayisah, K.D.; Banito, A.; Gumedzoe, Y.M.D. Criblage de clones d'ignames du complexe *Dioscorea cayenensis-rotundata* pour la résistance au virus de la mosaïque de l'igname (YMV) au Togo. *Int. J. Biol. Chem. Sci.* **2014**, *8*, 2198–2206. [CrossRef]
46. Dafni, A.; Kevan, P.G.; Husband, B.C. *Practical Pollination Biology Enviroquest*; Enviroquest, Ltd.: Cambridge, ON, Canada, 2005.
47. Shivanna, K.R. Reproductive assurance through autogamous self-pollination across diverse sexual and breeding systems. *Curr. Sci.* **2015**, *109*, 1255–1263. [CrossRef]
48. Yacouba, B.; Michel, K.A.; Brice, D.K.E.; Emmanuel, E.A.; Sidoine, E.B.; Abou Bakari, K.; Patrik, A. Effet de la Période de Pollinisation Manuelle sur la Fructification de l'igname (*Dioscorea rotundata* Poir; Dioscoreaceae) en Côte d'Ivoire. *Europ. Sci. J.* **2019**, *15*, 1857–7881. [CrossRef]
49. Jacquemart, A.-L.; van der Michotte, A.A.; Raspe, O. Compatibility and pollinator efficiency tests on *Pyrus communis* L. cv. "Conference". *J. Hortic. Sci. Biotechnol.* **2006**, *81*, 827–830. [CrossRef]
50. Ahoton, L.E.; Adjakpa, J.B.; Gouda, M.; Daînou, O.; Akpo, E.L. Effet des prétraitements de semences du prunier des savanes (*Vitex doniana* Sweet) sur la germination et la croissance des plantules. *Ann. Des. Sci. Agron.* **2011**, *15*, 21–35. [CrossRef]
51. Tiama, D. Diversité Génétique des Ignames *Dioscorea* sp. du Burkina Faso: YùYàdu Passoré. Ph.D. Thesis, Université Ouaga I Professeur Joseph KI-ZERBO, Ouagadougou, Burkina Faso, 2016.
52. Assaba, E.I.; Yolou, M.; Totin Vodounon, H.S.; Quenum, G.A.; Zoundjihekpon, J. Caractéristiques des graines d'ignames cultivées du complexe d'espèces *Dioscorea cayenensis—Dioscorea rotundata* et évolution du taux de germination des graines. *Afr. Sci.* **2018**, *14*, 195–208.
53. Lloyd, D.G.; Schoen, D.J. Self- and cross-fertlization in plants. *Int. J. Plant Sci.* **1992**, *153*, 358–369.
54. Sinébou, V.; Quinet, M.; Ahohuendo, B.C.; Jacquemart, A.-L. Reproductive traits affect the rescue of valuable and endangered multipurpose tropical trees. *AoB Plants* **2016**, *8*, plw051. [CrossRef]
55. Charlesworth, D.; Charlesworth, B. Inbreeding depression and its evolutionary consequences. *Ann. Rev. Syst. Ecol.* **1987**, *18*, 237–268. [CrossRef]
56. Kouakou, K.C. La Côte d'Ivoire à La Recherche d'Un Droit de la Consommation. Ph.D. Thesis, University of Montpellier I, Montpellier, France, 1987.
57. Bai, K.V.; Jos, J.S. Female fertility and seed set in *Dioscorea alata* L. *Trop. Agric.* **1986**, *63*, 7–10.
58. de Nettancourt, D. Incompatibility in angiosperms. *Mono Theor. Appl. Genet.* **1977**, *3*, 78–79.
59. Igic, B.; Lande, R.; Kohn, J.R. Loss of self-incompatibility and its evolutionary consequences. *Int. J. Plant Sci.* **2008**, *169*, 93–104. [CrossRef]
60. Muñoz-Sanz, J.V.; Zuriaga, E.; Cruz-García, F.; McClure, B.; Romero, C. Self-(In) compatibility systems: Target traits for crop-production, plant breeding, and biotechnology. *Front. Plant Sci.* **2020**, *11*, 195. [CrossRef] [PubMed]
61. Girma, G.; Natsume, S.; Carluccio, A.V.; Takagi, H.; Matsumura, H.; Uemura, A.; Tamiru, M. Identification of candidate flowering and sex genes in white Guinea yam (*D. rotundata* Poir.) by SuperSAGE transcriptome profiling. *PLoS ONE* **2019**, *14*, e0216912. [CrossRef] [PubMed]
62. Mondo, J.M.; Agre, P.A.; Asiedu, R.; Akoroda, M.O.; Asfaw, A. Genome-wide association studies for sex determination and cross-compatibility in water yam (*Dioscorea alata* L.). *Plants* **2021**, *10*, 1412. [CrossRef]
63. Myhal, M.D.; Mishchenko, S.V.; Laiko, I.M. *Inbreeding and Heterosis of Hemp*; Sumy Inc.: Sumy, Ukraine, 2020.
64. Mishchenko, S.V. Phenotypic manifestation of the traits of presence and content of cannabinoids in the process of self-pollination in monoecious hemp and selection. *Plant Breed. Seed Prod.* **2020**, *118*, 58–73. [CrossRef]
65. Faux, A.M.; Draye, X.; Lambert, R.; d'Andrimont, R.; Raulier, P.; Bertin, P. The relationship of stem and seed yields to flowering phenology and sex expression in monoecious hemp (*Cannabis sativa* L.). *Euro J. Agron.* **2013**, *47*, 11–22. [CrossRef]

66. Carlson, C.H.; Stack, G.M.; Jiang, Y.; Taskiran, B.; Cala, A.R.; Toth, J.A.; Philippe, G.; Rose, J.K.; Smart, C.D.; Smart, L.B. Morphometric relationships and their contribution to biomass and cannabinoid yield in hybrids of hemp (*Cannabis sativa*). *J. Exp. Bot.* **2021**, *72*, 7694–7709. [CrossRef]
67. Kurtz, L.E.; Mahoney, J.D.; Brand, M.H.; Lubell-Brand, J.D. Comparing genotypic and phenotypic variation of selfed and outcrossed progeny of hemp. *HortScience* **2020**, *55*, 1206–1209. [CrossRef]
68. Zhang, B.; Zhu, W.; Diao, S.; Wu, X.; Lu, J.; Ding, C.; Su, X. The poplar pangenome provides insights into the evolutionary history of the genus. *Comm. biol.* **2019**, *2*, 215. [CrossRef]
69. Schultz, S.T.; Ganders, F.R. Evolution of unisexuality in the Hawaiian flora: A test of microevolutionary theory. *Evolution* **1996**, *50*, 842–855. [CrossRef] [PubMed]
70. Dorken, M.E.; Friedman, J.; Barrett, S.C. The evolution and maintenance of monoecy and dioecy in *Sagittaria latifolia* (Alismataceae). *Evolution* **2002**, *56*, 31–41. [CrossRef]
71. Mondo, J.M.; Agre, P.A.; Edemodu, A.; Asiedu, R.; Akoroda, M.O.; Asfaw, A. Cross compatibility in intraspecific and interspecific hybridization in yam (*Dioscorea* spp.). *Sci. Rep.* **2022**, *12*, 3432. [CrossRef] [PubMed]
72. Ehounou, A.E.; Kouakou, A.M.; N'zi, J.C.; Dibi, K.B.E.; Bakayoko, Y.; Essis, B.S.; N'Guetta, A.S.P. Production of hybrid seeds by intraspecific crossing in yam *Dioscorea alata* L. *Int. J. Sci. Res.* **2019**, *8*, 1212–1221.
73. Mignouna, H.; Mank, R.; Ellis, T.; Van Den Bosch, N.; Asiedu, R.; Abang, M.; Peleman, J. A genetic linkage map of water yam (*Dioscorea alata* L.) based on AFLP markers and QTL analysis for anthracnose resistance. *Theor. Appl. Genet.* **2002**, *105*, 726–735. [CrossRef] [PubMed]
74. Agbangla, C. Genetic structure of farmer-managed varieties in clonally propagated crops. *Genetica* **2011**, *139*, 1055–1064.
75. Tostain, S.; Scarcelli, N.; Brottier, P.; Marchand, J.L.; Pham, J.L.; Noyer, J.L. Development of DNA microsatellite markers in tropical yam (*Dioscorea* sp.). *Mol. Ecol. Notes* **2006**, *6*, 173–175. [CrossRef]
76. Obidiegwu, J.E.; Kolesnikova-Allen, M.; Ene-Obong, E.E.; Muoneke, C.O.; Asiedu, R. SSR markers reveal diversity in Guinea yam (*Dioscorea cayenensis/Dioscorea rotundata*) core set. *Afr. J. Biotechnol.* **2009**, *8*, 2730–2739.
77. Scarcelli, N. Structure et Dynamique D'une Plante Cultivée A Multiplication Végétative: Le cas Des Ignames Au Bénin (*Dioscorea* sp.). Ph.D. Thesis, Biologie des Systèmes Intégrés, Agronomie-Environnement, University of Montpellier II, Montpellier, France, 2005. Available online: http://www.documentation.ird.fr/intranet/publi/depot/2009-10-13/010035303.pdf (accessed on 24 September 2021).
78. Bhattacharjee, R.; Agre, P.; Bauchet, G.; De Koeyer, D.; Lopez-Montes, A.; Kumar, P.L.; Abberton, M.; Adebola, P.; Asfaw, A.; Asiedu, R. Genotyping-by-sequencing to unlock genetic diversity and population structure in White yam (*Dioscorea rotundata* Poir.). *Agronomy* **2020**, *10*, 1437. [CrossRef]
79. Rudi, N.; Norton, G.W.; Alwang, J.R.; Asumugha, G.N. Economic impact analysis of marker-assisted breeding for resistance to pests and post harvest deterioration in cassava. *Afr. J. Agric. Resour. Econ.* **2010**, *4*, 110–122.
80. Perera, P.I.; Quintero, M.; Dedicova, B.; Kularatne, J.; Ceballos, H. Comparative morphology, biology and histology of reproductive development in three lines of Manihot esculenta Crantz (Euphorbiaceae: Crotonoideae). *AoB Plants* **2013**, *5*, pls046. [CrossRef] [PubMed]
81. Bull, S.E.; Alder, A.; Barsan, C.; Kohler, M.; Hennig, L.; Gruissem, W.; and Vanderschuren, H. Flowering Locus T triggers early and fertile flowering in glasshouse cassava (*Manihot esculenta* Crantz). *Plants* **2017**, *6*, 22. [CrossRef]
82. Ceballos, H.; Jaramillo, J.; Salazar, S.; Pineda, L.M.; Calle, F.; Setter, T. Induction of flowering in cassava through grafting. *J. Plant Breed. Crop Sci.* **2017**, *9*, 19–29.
83. Hyde, P.T.; Guan, X.; Abreu, V.; Setter, T.L. The anti-ethylene growth regulator silver thiosulfate (STS) increases flower production and longevity in cassava (*Manihot esculenta* Crantz). *Plant Growth Regul.* **2020**, *90*, 441–453. [CrossRef]
84. Mukherjee, A.; George, J.; Pillai, R.; Chakrabarti, S.K.; Naskar, S.K.; Patro, R.; Nayak, S.; Lebot, V. Development of taro (*Colocasia esculenta* (L.) Schott) hybrids overcoming its asynchrony in flowering using cryostored pollen. *Euphytica* **2016**, *212*, 29–36. [CrossRef]
85. Muthoni, J.; Shimelis, H.; Melis, R.; Kabira, J. Reproductive biology and early generation's selection in conventional potato breeding. *Aust. J. Crop Sci.* **2012**, *6*, 488–497.
86. Orozco-Arroyo, G.; Vázquez-Santana, S.; Camacho, A.; Dubrovsky, J.G.; Cruz-García, F. Inception of maleness: Auxin contribution to flower masculinization in the dioecious cactus Opuntia stenopetala. *Planta* **2012**, *236*, 225–238. [CrossRef] [PubMed]
87. Souza, L.S.; Diniz, R.P.; de Jesus Neves, R.; Alves, A.A.C.; de Oliveira, E.J. Grafting as a strategy to increase flowering of cassava. *Sci. Hortic.* **2018**, *240*, 544–551. [CrossRef]
88. Mondo, J.; Agre, P.; Edemodu, A.; Adebola, P.; Asiedu, R.; Akoroda, M.; Asfaw, A. Floral Biology and Pollination Efficiency in Yam (*Dioscorea* spp.). *Agriculture* **2020**, *10*, 560. [CrossRef]
89. Mondo, J.M.; Agre, P.A.; Asiedu, R.; Akoroda, M.O.; Asfaw, A. Optimized Protocol for In Vitro Pollen Germination in Yam (*Dioscorea* spp.). *Plants* **2021**, *10*, 795. [CrossRef]