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Pollination Success in Some White Yam Genotypes Under Polycross and Nested Mating Designs

Prince Emmanuel Norman[1, 2, 3, *], Asrat Asfaw[2, *], Pangirayi Bernard Tongoona[3], Agyemang Danquah[3], Eric Yirenkyi Danquah[3], David De Koeyer[2], Robert Asiedu[2]

[1]Sierra Leone Agricultural Research Institute (SLARI), Freetown, Sierra Leone
[2]International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria
[3]West Africa Centre for Crop Improvement (WACCI), College of Basic and Applied Sciences, University of Ghana (UG), Legon, Ghana

Email address

pnorman@wacci.edu.gh (P. E. Norman), a.amele@cgiar.org (A. Asfaw)

[*]Corresponding author

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Abstract: Breeding yams is a two-step process that combines sexual and asexual reproduction. Sexual reproduction involving a transfer of pollen from male to female genotype reproductive organ is a key step to generate variability in yam improvement. The efficacy of genetic improvement therefore depends on degree of pollination success resulting in fruit set and producing viable botanical seeds. This study assessed pollination success attributes in white yam (*Dioscorea rotundata*) genotypes using two mating designs: polycross and nested mating designs. Selected floral, fruit and seed traits were assessed in 12 parents (comprising nine females and three males) used in a polycross (natural) and nested (artificial hand pollination) mating designs. Total fruit sets and number of filled seeds were lower under nested mating compared to the polycross design. However, filled seeds per fruits were higher in nested mating than in polycross design. Fruiting success and number of flowers pollinated contributed more to the variability in filled seeds in polycross (R^2=0.67; $p<0.05$) than nested mating (R^2=0.40; p=0.301). Mean kinship (MK) values showed weak positive association with fruit set per plant in both NC-I (r=0.312) and polycross (r=0.05) designs. The relationship between flowering intensity and pollination success was high and positive in polycross (r=0.85), but low and negative in NC-I (r=-0.06). Genome uniqueness had weak association with fruit set success NC-I (r=0.23) and polycross (r= -0.014) designs. The results suggest complementarity of polycross and nested mating designs for optimizing population improvement and variety development in white yam breeding.

Keywords: Pollination Success, *Dioscorea rotundata* Poir, Parental and Pedigree Relationships, Reproductive Trait Associations, Mating Designs

1. Introduction

Yam (*Dioscore*a spp.) is a multi-species crop cultivated around the world for its underground starchy tubers [1]. Among the many cultivated yam species, white yam or Guinea yam (*Dioscorea rotundata* Poir) is an important source of food and income for millions of smallholders in West Africa where 93% of the global yam production occurs [2]. Yam also holds an eminent social and cultural position in West African agri-food system. Socio-culturally, the crop is used to grace marriage, naming, burial, ritual and other traditional purposes [3]. Despite its importance, yam has not been improved for decades in terms of its yield or productivity gains. Number of factors contributes to lack of productivity gains in this highly prized African food crop. Availability and access to more productive, profitable and resilient yam varieties responding to climate change risks, and meeting the producers and consumers demand is among the factors limiting productivity gain in yams. Development and deployment of improved varieties is therefore among the action steps to maximize the productivity gains and lift the crop's value to the society. Yam variety development/breeding is a two-step process that combines both sexual and asexual reproductions. Pollination is a key

sexual reproduction process to generate variability and make genetic improvement in yams. The rate of pollination success under different mating systems is most important for optimizing genetic improvement in crops [4]. Such knowledge can help to determine the manipulation space available to accomplish crop improvement.

Shy flowering or lack of flowering synchronization of many genotypes, cross incompatibility, the predominantly dioecious state of most flowering yam genotypes and the erratic climatic impact on growth and reproduction contribute to crossing outcomes of different mating systems [5, 6]. Fruit and seed sets are influenced by the sex ratio of the population, spatial distribution of male and female plants within the population of dioecious species, flowering intensity [7, 8, 9, 10] and behavior of pollinators [11, 12]. The existence of different sex ratios in yams including dioecious, monoecious, or hermaphrodite types [10], and the varying flowering patterns of genotypes (no flowering, sparse and profuse flowering) also limit the amount of fruit and seed sets in compatible genotypes [10]. Yam flowering and fruit sets may also be affected by the vigour of the plant, severity of pest and disease attacks [5].

Various studies have been conducted on the reproductive biology of tropical yam species [8, 10, 13, 14, 15]. In plants, reproductive biology involves flowering phenology, floral biology, pollen-pollinator interaction, mating systems and gene flow via pollen and seeds [6]. Reproductive biology is a useful phenomenon in genetic improvement, conservation and utilization of crop biodiversity. A good understanding of flowering duration of female plants, flowering intensity, fruit and seed set under different mating systems in yams is vital to guide and complement the development of genotypes using natural and/or controlled pollination systems. However, little comparative studies on mating systems have been done for the determination of the system with better fruit set, seed set and botanical seed yield production in yams. Moreover, there is lack of information on level of existing variations within seed dimensions and colors in *D. rotundata*.

The aim of this study was to assess the degree of pollination success under two mating schemes and the effect of parental pedigree relationship on pollination success in white yam genotypes.

2. Materials and Methods

2.1. Experimental Site

The study was conducted at the experimental site of the International Institute of Tropical Agriculture (IITA), Ibadan campus, Nigeria during 2016/2017 cropping season. The site represents a transition rainforest zone and receives annual rainfall of 1554.35 mm, mean minimum and maximum air temperature of 22.7 and 31.8°C, and mean minimum and maximum relative humidity of 51.5 and 95.7%, respectively, based on weather data compiled from the Geographical Information System (GIS), IITA, Ibadan station, Nigeria. The crossing blocks were planted separately at about 700 m apart with different geographic coordinates. The female crossing block was laid out in an area with 7°30.228"N latitude, 3°54.143"E longitude and an elevation of 238 m a.s.l.; the male crossing block was established in area with 7°29.382"N latitude, and 3°54.449"E longitude at 226 m a.s.l.; and the polycross block was planted in an isolation plot located at 7°29.098"N latitude and 3°54.125"E longitude at 210 m a.s.l.

The soils from the crossing blocks are well to moderately well-drained sandy-loam. The fresh subsamples were randomly collected in each crossing block at 0-20 cm soil depth prior to planting using a Jarret T-handle soil auger (100 mm head diameter) and bulked into composite sample per trial site.

Soil analyses were done using the procedures described by the International Soil Reference and Information Center (ISRIC) and the FAO [16]. Soil color was visually compared with the Munsell Chart while pH was determined on 1:1 soil: water extracts. Organic carbon was done by titration following the Walkley-Black technique, while nitrogen was determined by Kjeldahl distillation. Available P was determined using the Bray 1 procedure, while exchangeable potassium (K) was measured on neutral 1N ammonium acetate extracts and read on a Flame Photometer. Exchangeable Acidity (Al + H) was extracted by 1M KCl and titrated with 0.025 M NaOH.

The soil attributes of the crossing blocks are presented in Table 1. Generally, the soils at the trial sites were slightly acidic with the male crossing block exhibiting highest C:N ratio, P and K contents, whereas the polycross block had the highest organic matter and organic carbon contents.

Table 1. *Soil physical and chemical attributes of the female, male and poly- crossing blocks.*

Crossing block (CB)	pH (H_2O) 1:1	%OC	OM	%N	C/N Ratio	Bray P (mg/kg)	K (Cmol+/kg)
Female CB	6.0	0.13	0.22	0.01	11.64	2.61	0.23
Male CB	6.3	0.20	0.34	0.01	17.20	15.10	0.23
Polycross B	6.3	0.28	0.48	0.02	14.49	4.77	0.20

The experimental site was first ploughed, harrowed to break large clod soils, ridged and then laid out prior to planting material preparation and planting.

2.2. Plant Material, Mating Designs and Crop Management Practices

Twelve genotypes of *D. rotundata* comprising three males and nine females with desired complementary traits for fresh tuber, dry matter, tuber shape, as well as earliness and tolerance to yam mosaic virus and anthracnose were used (Table 2).

Two mating schemes, an open natural pollination in a polycross (PC) and a controlled artificial hand pollination in a nested mating (North Carolina design I) were used. The mating schemes used targeted crossing of three female parents to a male parent (3:1) producing nine cross

combinations of the studied genotypes. In the nested mating or North Carolina design I (NC-I), male and female crossing blocks were planted in an isolation blocks. The female parents were hand-pollinated with pollens extracted from anthers of male parents by hocking them to the stigma of the newly opened female flowers. Prior to pollination, young unopened female flower buds were protected by bagging for about 3 to 7 days to prevent insect-pollination [17]. After pollination, the female flowers were again bagged to avoid contamination by unwanted pollens. The bags were removed after 2-3 weeks to permit congenial fruit development. The polycross field was simulated the NC-I mating plan where the genotypes were grown in plant hills arranged in a balanced neighbor effect design that ensures equal chance pollination of female plants by its intended male pollinator.

In both mating designs, healthy tubers of each genotype were cut into setts of 250 g each, pre-treated in a mixture of 70 g Macozeb, 75 mL Chlorpyrifos and 10 L tap water for 5 min, dried for 20h under shade prior to planting in holes made on the crest of mounds. In the NC-I design, male and female plants were planted sequentially in single row of 10 plants plot at a spacing of 1 m x 1 m on two dates: the first planting was on the 9th April 2016 for both male and female crossing blocks; the second planting of the female crossing block was on the 30th April 2016, whilst the male crossing block was established on the 2nd May 2016. In the polycross, both male and female parents were planted on the 2nd May 2016 using similar plant spacing. A total of 4 setts per female genotype and 28 setts per male genotype were used. The crossing blocks were managed using the standard yam husbandry practices, except that no fertilizer and pesticide were applied [18].

Table 2. *Accession numbers, status, sexes and attributes of Dioscorea rotundata genotypes used in the study.*

Accession no.	Status	Sex	Attributes
TDr97/00793	Improved variety	Female	mild to low YMV, mild YAD, high flowering, oval tubers with few spines, hairs and wrinkle on tuber surface, medium dry matter
TDr89/02157	Improved variety	Female	mild to low YMV, mild YAD, irregular tubers with few spines, hairs and wrinkle on tuber surface, high yield and dry matter
TDr89/02475	Improved variety	Female	mild to low YMV, mild YAD, irregular tubers with few spines, hairs and wrinkle on tuber surface, medium dry matter
TDr97/00632	Improved variety	Female	low to moderate YMV, mild to low YAD, earliness, oval oblong, smooth tubers with few spines, hairs and wrinkle on tuber surface, multiple tubering, medium dry matter, good cooking quality
TDr97/00205	Improved variety	Female	mild to low YMV, mild YAD, earliness, oval tubers with few spines, hairs and wrinkle on tuber surface, multiple tubering, medium dry matter, good cooking quality
TDr08-21-3 (Ekpe-II)	Adapted variety	Female	low to moderate YMVS, mild to low YAD, earliness, oval oblong, smooth tubers with few spines, hairs and wrinkle on tuber surface, multiple tubering, medium dry matter, good cooking quality
TDr95/19158	Improved variety	Female	mild to low YMV, mild YAD, fair flowering, palmitic tubers with few spines, hairs and wrinkle on tuber surface, highly oxidizing, medium dry matter
TDr95/18988	Improved variety	Female	mild to low YMV, mild YAD, low flowering, oval tubers with few spines, hairs and wrinkle on tuber surface, NUE, medium dry matter
TDrOjuiyawo	Adapted landrace	Female	mild to low YMV and YAD, oval tuber shape, smooth tubers with few spines, hairs and wrinkle on tuber surface, medium dry matter, good cooking quality
TDr99/02789	Improved variety	Male	low to moderate YMV, mild to low YAD, high flowering, oval tubers with intermediate spines, few hairs and wrinkle on tuber surface, drought tolerant, oxidizing.
TDr99/02607	Improved variety	Male	mild to low YMV, mild YAD, oval oblong tubers with intermediate spines, hairs and wrinkle on tuber surface, high flowering, RVA value for starch, slightly oxidizing.
TDr95/01932	Improved variety	Male	mild YMVS and YADS, oval oblong, smooth tubers with few spines, hairs and wrinkle on tuber surface, high flowering, multiple tubering, slightly oxidizing, high dry matter, good cooking quality.

TDr = Tropical *Dioscorea rotundata*, YMV = yam mosaic virus; YAD = yam anthracnose disease

2.3. Data Collection and Analysis

Data on selected reproductive traits were collected using procedures described in yam ontology (https://yambase-test.sgn.cornell.edu/breeders/phenotyping) and standard operating protocol for yam [18] with slight modifications. The pollination success attributes measured included number of flowers produced per plant (count), number of spikes per plant (count), spikes pollinated per plant, number of flowers pollinated per plant (count), fruit set per plant (count) and fruiting index (calculated as number of fruit set per plant divided by number of pollinated flowers per plant). The flowering window of genotypes was determined as the date spanning from the first pollination to the last pollination. Number of flowers, fruits and seeds per plant were done relative to number of sprouted plants [10]. Fruit development was visibly noted within two weeks, with fruit counts recorded after two weeks of last date of pollination. Data on seed characteristics (seed length, width and area) were captured with imaging system in 1KK version 1.0 App.

Data collected on numbers of spikes, flowers, fruits, seeds and their derived ratios were analyzed using the General Linear Model procedure (PROC GLM) of SAS version 9.4 [19]. The simple linear regression and multiple linear regression analyses were done for determination of seed set in each mating system. The simple linear regression equations used were:

$$Y = \beta_0 + \beta_{POLS}X_{POLS}$$

$$Y = \beta_0 + \beta_{NFLP}X_{NFLP}$$

The multiple linear regression equation used was:

$$Y = \beta_0 + \beta_{POLS}X_{POLS} + \beta_{NFLP}X_{NFLP}$$

Where, Y=proportion of ovules setting filled seeds (POSFS), determined as the proportion of filled seeds per flowers pollinated; β_0=intercept; X_{POLS}=pollination success (POLS), determined as the proportion of fruit set from pollinated flowers per plant; and X_{NFLP}=mean ovule production per plant (number of flowers produced per plant) (NFLP). We also employed pedigree matrix analysis to assess genetic structure and diversity of the yam genotypes in the study using R (statistical software).

3. Results

3.1. Flowering Window and Pollination

The flower bud initiation in NC-I started on the 18th June 2016, whilst in the polycross, it started on 15th June 2016. Bagging and pollination in NC-I started on 21st July 2016 and 27th July 2016, respectively, with the last pollination done on 15th September 2016. The number of flowers pollinated in every two weeks varied among genotypes. Genotype TDrOju-iyawo exhibited highest number of flowers pollinated per plant (123 flowers) (Table 3; Figure 2) with pollination lasting from the 3rd to the 8th weeks from the date of first flower initiation. Genotype TDr97/00793 had the longest time of pollination (flowering window) spanning from the 1st week to the 8th week from the first flower initiation in a plant. Genotype TDr08-21-3 (Ekpe II) had the narrowest flowering window and was among genotypes with lowest flowers pollinated per plant. In the polycross system, TDr08-21-3 (Ekpe II) was much late flowering producing fewer flowers per plant resulting into no fruit formation (Table 3; Figure 2). The results imply that the flowering window or pollination duration and flowering intensity of each genotype are among factors that serve as a guide for selection of trait progenitors in population development.

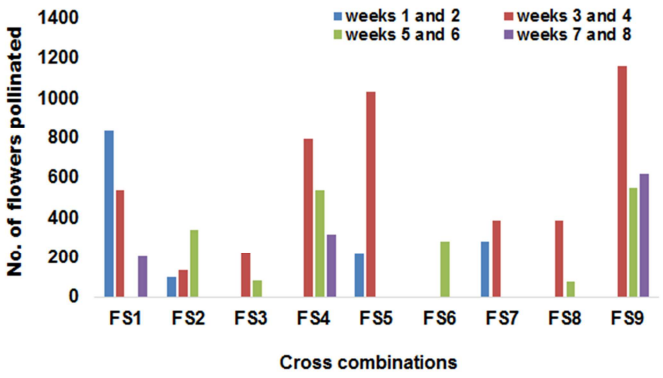

Figure 1. *Variation in flowering duration and floral pollination among the female parents in NC-I mating system. FS1=TDr97/00793 × TDr99/02789; FS2=TDr89/02157 × TDr99/02789; FS3=TDr89/02475 × TDr99/02789; FS4=TDr97/00632 × TDr99/02607; FS5=TDr97/00205 × TDr99/02607; FS6=TDr08-21-3 × TDr99/02607; FS7=TDr95/19158 × TDr95/01932; FS8=TDr95/18988 × TDr95/01932; and FS9=TDrOjuiyawo × TDr95/01932.*

Table 3. *Mean number of spikes pollinated per plant (NSPP), flowers per spikes (NFLS), flowers per plant (NFLP), flowers pollinated per plant (NFLPP), fruit set per plant (NFRSP) and fruiting index (FI) of nine female parents of D. rotundata in NC-I and polycross mating systems.*

	NSPP	NFLS	NFLP	NFLPP	NFRSP	FI*
Crossing parents	North Carolina Design I					
TDr97/00793 × TDr99/02789	10.6	7.9	84.3	80.5	4.9	0.06
TDr89/02157 × TDr99/02789	2.8	8.5	23.5	23.1	2.7	0.11
TDr89/02475 × TDr99/02789	2.1	8.4	17.2	15.7	2.8	0.16
TDr97/00632 × TDr99/02607	13.1	8.1	105.8	97.1	41.9	0.40
TDr97/00205 × TDr99/02607	6.2	10.4	63.8	58.4	15.9	0.25
TDr08-21-3 × TDr99/02607	2.0	8.2	16.5	16.4	5.5	0.34
TDr95/19158 × TDr95/01932	3.9	10.5	40.9	39.2	19.4	0.47
TDr95/18988 × TDr95/01932	1.9	9.7	18.4	18.4	4.7	0.26
TDrOju-iyawo × TDr95/01932	5.8	23.1	133.7	122.6	24.7	0.18
Mean	5.4	10.5	56.0	52.4	13.6	0.25
SE	1.4	1.6	14.5	13.2	4.4	0.05
	Polycross Design					
TDr97/00793	27.8	7.0	193.0	193.0	66.5	0.34
TDr89/02157	13.0	6.4	82.8	82.8	45.5	0.55
TDr89/02475	37.5	8.7	327.5	327.5	117.5	0.36
TDr97/00632	29.0	5.4	157.5	157.5	39.3	0.25
TDr97/00205	58.0	8.1	468.3	468.3	270.3	0.77
TDr08-21-3	0.7	2.5	1.7	1.7	0.0	0.00
TDr95/19158	1.5	7.3	11.0	11.0	6.3	0.57
TDr95/18988	8.5	6.0	51.3	51.3	30.5	0.60
TDrOju-iyawo	107.5	6.8	729.5	729.5	373.8	0.51
Mean	31.49	6.5	224.7	224.7	115.5	0.44
SE	11.34	0.6	81.3	81.3	48.91	0.08

*FI is also referred to as pollination success (POLS); SE = Standard error

3.2. Pollination Success, Seed Attributes and Relationships

Generally, the stepwise regression of proportion of ovules setting filled seeds indicated that pollination success contributes more to fruit and seed set than the number of flowers pollinated per plant (Figure 2). Moreover, the multiple regression analysis indicates that both pollination success and number of flowers pollinated per plant contributed more to the variability in filled seed formation in polycross (R^2=0.67; p<0.05) than NC-I (R^2=0.40; p=0.301) (Table 4).

In the NC-I mating system, the increased filled botanic seed set (formation) relates with increased pollination success and number of available flowers that were successfully pollinated per plant. Pollination success and number of flowers produced per plant accounted for 39.9% of total variability in seed set (R^2=0.40; p=0.301). The result implies that the remaining percent variability is possibly attributed to damage to the stigmatic surface forming seeds during pollination, higher aborted fruit development, erratic climatic condition at time of pollination, among other factors. Damage to stigmatic surface by pollinating needle or lack of adherence of pollen to the stigmatic surface contributes to reduced pollen germination on stigmatic surface and growth down the style. However, flowers that are successfully pollinated produced higher numbers of filled seeds per fruit compared to those in the polycross system (Table 5).

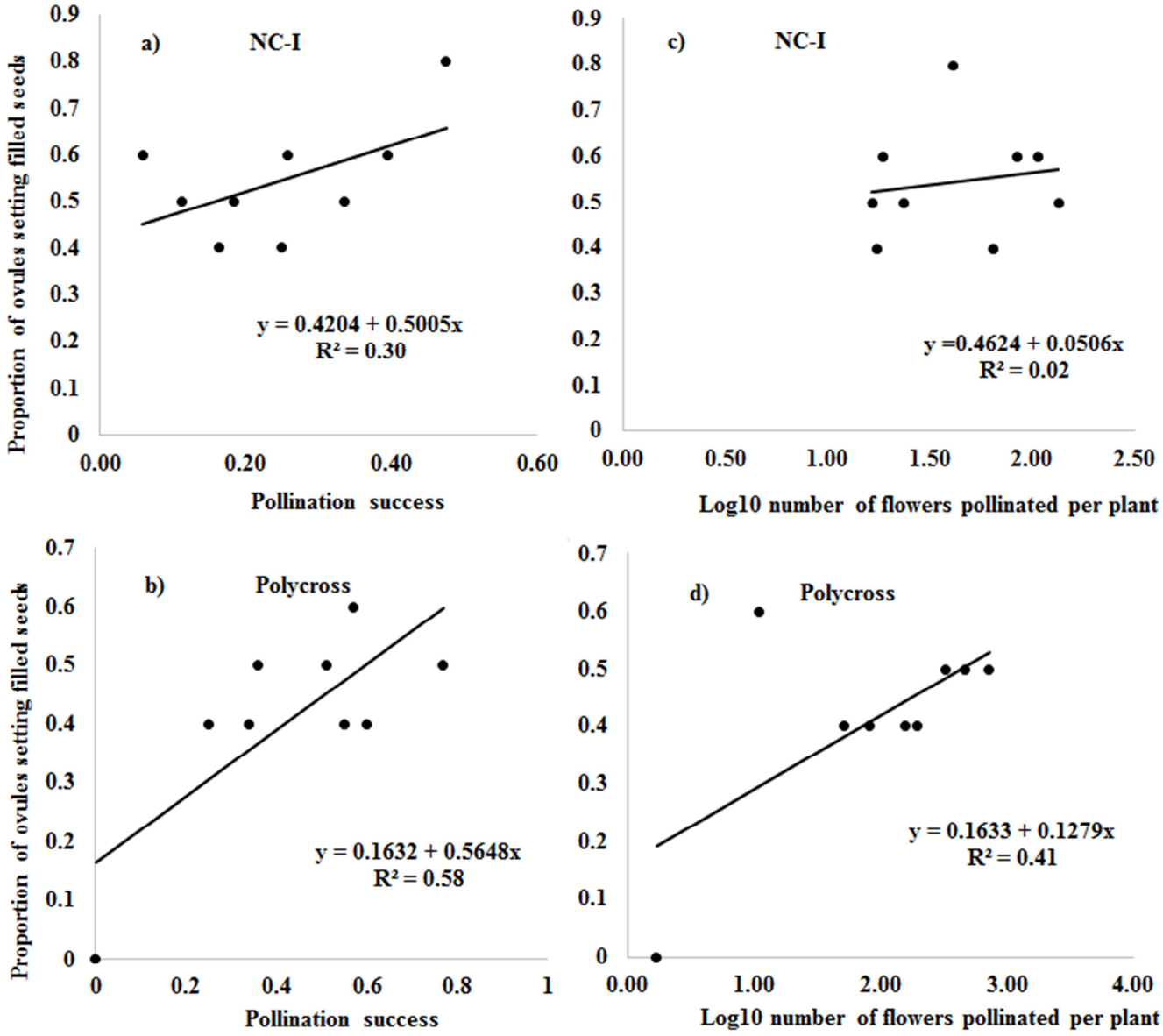

Figure 2. *Relationships between proportion of ovules setting filled seeds and pollination success (a and b); and between proportion of ovules setting seeds and Log10 number of flowers pollinated per plant (c and d), in NC-I and polycross, respectively. The x axis=pollination success (a and b); x axis=Log10 number of flowers pollinated per plant (c and d); and y axis= proportion of ovules setting seeds.*

Table 4. *Summary of relationships, of flower, fruit and seed formation of nine genotypes in NC-I and polycross mating systems.*

Mating design	Multiple linear regression (POSFS on POLS + NFLP)	Coefficient of determination	p-value
NC-I	$y = 0.332 + 0.502X_1 + 0.061X_2$	$R^2 = 0.399$	P = 0.301
Polycross	$y = 0.087 + 0.437X_1 + 0.069X_2$	$R^2 = 0.665$	P < 0.05

POSFS = proportion of ovules setting seeds; POLS = pollination success; and NFLP = number of flowers produced per plant

Table 5. *Number of aborted seeds per plant (NASP), filled seeds per plant (NFSP), filled seeds per fruit (NFSF), total seeds per fruit (NTSF) and filled seeds per total seeds ratio (NFSTSR) of nine female parents of D. rotundata in NC-I and polycross mating systems.*

	NASP	NFSP	NFSF	NTSF	NFSTSR*
Crossing parents	North Carolina Design I				
TDr97/00793 × TDr99/02789	8.1	13.7	2.8	4.4	0.6
TDr89/02157 × TDr99/02789	6.1	6.5	4.2	4.7	0.5
TDr89/02475 × TDr99/02789	8.6	6.1	2.3	5.4	0.4
TDr97/00632 × TDr99/02607	77.2	124.4	3.0	4.8	0.6
TDr97/00205 × TDr99/02607	43.6	30.9	2.0	4.8	0.4
TDr08-21-3 × TDr99/02607	11.8	9.5	1.7	3.9	0.5
TDr95/19158 × TDr95/01932	16.0	83.4	4.3	5.2	0.8
TDr95/18988 × TDr95/01932	11.1	16.2	3.0	5.8	0.6
TDrOju-iyawo × TDr95/01932	57.8	68.5	2.8	5.2	0.5
Mean	26.7	39.9	2.9	4.9	0.6
SE	8.7	14.1	0.3	0.2	0.04
	Polycross Design				
TDr97/00793	175.0	125.0	1.9	4.5	0.4
TDr89/02157	163.3	87.5	1.9	5.5	0.4
TDr89/02475	326.0	300.0	2.6	5.3	0.5
TDr97/00632	83.3	63.3	1.6	3.7	0.4
TDr97/00205	814.5	659.7	1.8	4.1	0.5
TDr08-21-3	0.0	0.0	0.0	0.0	0.0
TDr95/19158	15.3	19.5	3.1	5.6	0.6
TDr95/18988	94.5	60.5	2.0	5.1	0.4
TDrOjuiyawo	808.3	775.5	2.1	4.2	0.5
Mean	275.5	232.3	1.9	4.2	0.4
SE	106.2	96.6	0.3	0.6	0.05

*NFSTSR is also referred to as proportion of ovules setting seeds (POSFS); SE = Standard error

In the polycross design, pollination success and mean ovule produced per plant significantly accounted for 66.5% of total variability in seed set (R^2=0.67; p<0.05) (Table 5). The proportion of ovules forming filled botanic seeds may produce lower filled seeds per fruit relative to increased pollination success and mean number of flowers produced per plant. It is highly probable that low flowering intensity, lack of synchronization of flowering and pollinator participation in sufficiently loading pollen on stigma may have contributed most to the reduced seeds per fruit. The coefficient of determination of proportion of ovules forming filled botanic seeds versus number of flowers pollinated per plant was lower in NC-I (R^2=0.02) than polycross (R^2=0.41) (Figure 2. c, d). The results indicate that number of flowers pollinated per plant accounted for 2 and 41% of total variability in seed set of NC-I and polycross, respectively.

The mean seed area dimensions of the various genotypes ranged between 109.39 and 207.83 mm^2 (NC-I) and between 58.64 and 202.59 mm^2 (polycross design) (Table 6). In NC-I, genotype TDr89/02475 exhibited the largest seed area (207.83 mm^2) followed by TDr97/00793 (201.74 mm^2), while TDr97/00632 (109.39 mm^2) had the smallest. In the polycross, genotype TDr97/00793 (202.59 mm^2) had the highest, followed by TDr89/02475 (157.07 mm^2), whilst TDr97/00632 (58.64 mm^2) had smallest seeds.

Table 6. *Botanical seed dimension attributes of nine female parents of D. rotundata in NC-I and polycross mating systems using 1KK App.*

	Total seeds sampled	Mean seed length (mm)	CV	Mean seed width (mm)	CV	Mean seed area (mm^2)	CV
Crossing	North Carolina Design I						
TDr97/00793 × TDr99/02789	34	21.33	0.55	15.08	0.26	201.74	0.30
TDr89/02157 × TDr99/02789	33	15.95	0.11	13.22	0.17	145.21	0.20
TDr89/02475 × TDr99/02789	37	18.21	0.04	16.50	0.07	207.83	0.11
TDr97/00632 × TDr99/02607	33	13.42	0.17	11.63	0.19	109.39	0.28
TDr97/00205 × TDr99/02607	33	15.92	0.10	13.37	0.09	149.55	0.18

	Total seeds sampled	Mean seed length (mm)	CV	Mean seed width (mm)	CV	Mean seed area (mm^2)	CV
TDr08-21-3 × TDr99/02607	30	16.59	0.19	14.38	0.22	170.06	0.32
TDr95/19158 × TDr95/01932	33	17.09	0.07	15.50	0.07	183.93	0.16
TDr95/18988 × TDr95/01932	33	18.50	0.79	15.93	0.88	139.71	0.37
TDrOju-iyawo × TDr95/01932	33	17.14	0.05	15.43	0.08	178.70	0.12
	Polycross Design						
TDr97/00793	40	17.69	0.16	15.14	0.23	202.59	0.29
TDr89/02157	24	15.81	0.06	13.49	0.08	154.75	0.12
TDr89/02475	45	16.03	0.06	13.94	0.08	157.07	0.13
TDr97/00632	40	10.62	0.15	8.24	0.18	58.64	0.29
TDr97/00205	46	14.73	0.08	12.36	0.10	131.45	0.17
TDr08-21-3	NA	NA	NA	NA	NA	NA	NA
TDr95/19158	31	20.67	0.71	12.22	0.39	138.01	0.47
TDr95/18988	41	15.58	0.10	13.91	0.11	151.71	0.18
TDrOjuiyawo	34	21.07	0.60	13.73	0.15	149.53	0.13

NA = not available; CV = coefficient of variation

Similarly, the wings and seeds of the studied genotypes exhibited different colors. Of the nine female genotypes, six genotypes (TDr97/00793, TDr89/02157, TDr97/00632, TDr97/00205, TDr08-21-3 and TDrOju-iyawo) had dark-brown seeds with brown wing; one genotype (TDr89/02475) had brown seeds with light brown wing; one genotype (TDr95/19158) had dark-brown seeds with light-brown wing; and one genotype (TDr95/18988) had dark-brown seeds with brown wing (Table 7). Besides the variations in seed dimensions of NC-I and polycross (Table 6), both systems exhibited similar wing and seed color attributes.

Table 7. *Botanical seed and wing color attributes of nine full-sibs of D. rotundata NC-I crosses.*

Crossing parents	Seed color attributes
TDr97/00793 × TDr99/02789	Large dark-brown seeds with brown wing
TDr89/02157 × TDr99/02789	Dark-brown seeds with brown wing
TDr89/02475 × TDr99/02789	Brown seeds with light brown wing
TDr97/00632 × TDr99/02607	Small dark-brown seeds with brown wing
TDr97/00205 × TDr99/02607	Dark-brown seeds with brown wing
TDr08-21-3 × TDr99/02607	Dark-brown seeds with brown wing
TDr95/19158 × TDr95/01932	Dark-brown seeds with light-brown wing
TDr95/18988 × TDr95/01932	Brown seeds with brown wing
TDrOju-iyawo × TDr95/01932	Dark-brown seeds with brown wing

3.3. Parental Pedigree Relationship and Its Effect on Pollination Success

Pedigree information on 12 genotypes along with the exceeded ancestor was used to assess the effect of pedigree based population genetic attributes on pollination success traits in white yam (Table 8; Figure 3). Low values of inbreeding (0%) and average relatedness coefficient (3.4%) associated with high gene diversity (96.6%) were observed in the current yam accessions used in the breeding population. The average genome uniqueness (the probability that the genotype contains founder alleles not present in any other single genotypes with respect to current group members) was 27.6%. These low population statistics values might be related with the low pedigree depth observed in the white yam breeding population. The parents in the current population are less related, on average, with each other.

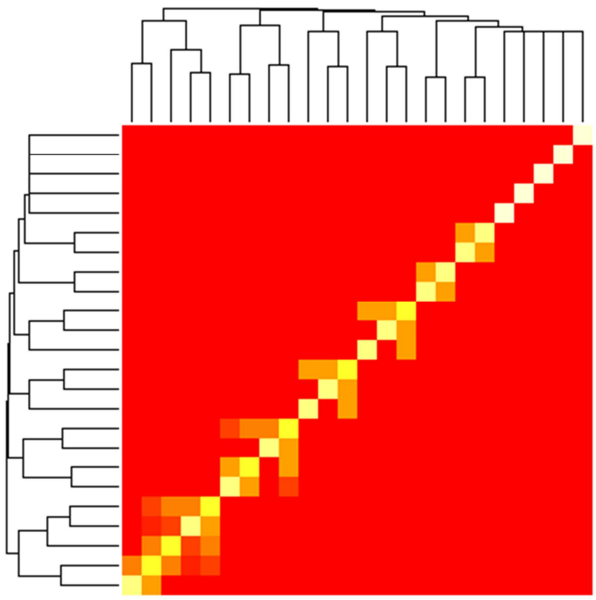

Figure 3. *Heat map showing population structure and diversity in the parents under current study using pedigree relationship matrix analysis.*

Table 8. *Population genetic statistics (inbreeding coefficient, mean kinship, genome uniqueness) from pedigree analysis and its effects on pollination success attributes (flowering intensity, fruit set) in 12 yam genotypes assessed in two mating systems: nested and polycross mating.*

Parents	Pedigree	Flower sex	F %	MK %	GU %	Pollination success attributes			
						NC-I		PC	
						FLRI	Fruit set per plant*	FLRI	Fruit set per plant*
TDr9700793	TDr93:23/Unknown	F	0	3.1	100	5	4.9	5	66.5
TDr8902157	Unknown	F	0	2.1	0	3	2.7	5	45.5
TDr8902475	Unknown	F	0	2.1	0	3	2.8	7	117.5
TDr9700632	TDr93:24/TDr8902494	F	0	4.2	100	5	41.9	5	39.3
TDr9700205	TDr8700839/TDr8700552	F	0	4.2	100	3	15.9	9	270.3
TDr08:21:3	Unknown	F	0	2.1	0	3	5.5	3	0
TDr9519158	Unknown	F	0	2.1	0	3	19.4	3	6.3
TDr9518988	TDr8700571/Unknown	F	0	3.1	100	3	4.7	3	30.5
TDrOjuiyawo	Unknown	F	0	2.1	0	3	24.7	9	373.8
TDr9902789	TDr93:50/TDr9502026 (TDr9100194/Unknown)	M	0	4.7	50	5	3.5	5	76.5
TDr9902607	TDr93:1/TDr9500858 (TDr8600309/Unknown)	M	0	4.9	50	7	21.1	7	103.2
TDr9501932	TDr8600309/Unknown	M	0	3.9	75.2	9	16.3	9	136.9

F = inbreeding coefficient; MK = mean kinship; GU = genome uniqueness; FLRI = flowering intensity; NC-I = North Carolina design I; PC = polycross. Fruit set for male genotypes was assumed as pollination of the male genotype with its female genotypes in crossing plan and the resulting fruit set in female genotypes crossed with the particular male in the plan calculated and taken as fruit set for males in involved in a crossing combination. So, the fruit set for males is assumed and calculated proxy guess not actual fruits set by males as male flowering diocese yams not produced fruits naturally.

None of the genotype in the current population has common ancestor. The mean relatedness or kinship coefficient (MK), determined as the mean of its kinship coefficient with every other member of the group including itself, of the genotypes in the current population ranged from 2.1% (those with unknown pedigree) to 4.9% (TDr9902607). The genome uniqueness ranged from 0% (those with unknown pedigree) to 100%. The pedigree based population genetic attributes has not shown any structured effect on pollination success in yam genotypes under the current population assessed in two mating systems. Mean kinship (MK) values revealed positive but weak association with fruit set per plant under both artificial hand pollination in nested mating design (r = 0.312) and open natural pollination in a polycross mating (r = 0.05). Flowering intensity exhibited high and positive association with pollination success under natural hybridization in a polycross mating (r = 0.85) but not under artificial hand pollination (r= -0.06). Genome uniqueness also show very weak association with fruit set success under controlled hand pollination in nested mating design (r = 0.23) and natural open pollination in a polycross mating (r= -0.014).

4. Discussion

The flowering window in the studied yam genotypes was relatively short (50 days), indicating the importance of effective and efficient plan for pollination of ready flowers with sufficiently viable pollens in a congenial pollination environment. Practices that promote higher flower production per plant is imperative so that even where fruit set is slightly limited by agents of flower abortion and pollen feeders, reasonable amounts of fruit and seed sets would be obtained. Moreover, flowering intensity, fruit and seed set also reflect the amount of nutrient resources supporting reproductive growth and development.

In the present study, natural pollination using polycross mating design resulted in a higher fruit set than the hand pollination in the NC design I. In the polycross design, reduced fruit set in genotypes was possibly due to low pollen loads on stigma, whilst in the nested mating under NC design I, reduced fruit set was possibly attributable to damage to stigmatic surface during pollination and/or use of pollen grains with reduced viability. Lack of flowering synchronization associated with the late flower bud initiation and flower opening in genotype TDr08-21-3 (Ekpe II) in a polycross mating might have resulted in pollination failure and no seed set. Genetic relatedness and inbreeding effects had no structure influence on the pollination success. However, flowering intensity significantly affected the pollination success most importantly under open and natural pollination systems. In general, our study revealed flowering intensity, flower synchronization and, successful transfer of pollen grains from male genotypes to stigma of female genotypes as important factors along with others that influence pollination success in yams. These findings are consistent with those reported by [10] and [20]. Moreover, the present study has unraveled the mating system that is more efficient in generating botanic seeds and established the parent-pedigree relationships which had never been done in yams.

In other crops, pollination success has been reported be influenced by pollen production, anther length, pollen grain size and mode of anther dehiscence [21, 22]. Additionally, pollen quantity and quality have been observed to influence the reproductive success in plants [23]. These aspects form part of future studies in yams.

Flowering time and duration, pollen availability and

viability, careful pollination to minimize damage to stigmatic surface, vigorous healthy status of crossing parents are known to be among critical factors that determine pollination success, fruit and seed sets in *Dioscorea rotundata* [5]. Pollination success and number of filled seeds per plant are lower in NC-I than in polycross; but filled seeds per successful fruit were higher in NC-I than in polycross. Findings indicated the great potential of polycross in generating high populations of yam progenies with enormous advantages including reduction of productivity loss, cheaper establishment costs (i.e. lacks costs of pollen collection, labeling, pollination materials, bagging, and pollination), no damage to stigmatic surfaces, etc. Optimizing polycross under open natural pollination is a potential mating strategy in yams for breeding as yam flowers are small and fragile making them difficult for artificial hand-pollination. The sticky nature of the male pollen also favors pollination by wind and insects in a polycross system. With the advent of DNA fingerprinting, it is possible to reconstruct the pedigree in the polycross mating for development of full and half-sib progenies with known parentages. Integrating parentage analysis with polycross mating as a breeding technique could enhance the improvement of pollination success and generation of large number of hybrid seeds with correct ancestry to fast track the genetic gain in yams.

The dimensions and colors of botanical seeds varied among the genotypes used in the study. Unlike other crops such as legumes and cereals where the market determines the seed attributes to breed for its genetic improvement, botanic seeds of yams have no market value instead of being used for breeding purposes as a source of variability for making the genetic improvement.

The pedigree based genetic relationship assessment could be an important aspect to maintain genetic diversity in a breeding population to safe guard varieties from biotic and abiotic stress but may not directly influence pollination success in yams. There may be other factors such as parental genetic compatibility, flowering efficiency, flower fertility, pollination agents (pollinator efficiency) and weather factors that could affect the pollination success in yams.

5. Conclusions

This study demonstrated that the polycross mating is more efficient in fruit setting (successful pollination) compared to the controlled pairwise system. Moreover, a good understanding of the flowering synchronization gap of each crossing parent enables effective implementation of sequential planting leading to higher pollination, fruit and filled seed sets. The variability in the proportion of filled seeds per plant in both systems depends more on pollination success than number of flowers produced per plant.

Authors' Contributions

AA and PEN designed the experiment. PEN, AA, PBT, DDD, EYD, AD and RA supervised the work. PEN and AA performed the data analysis and drafted the manuscript. All the authors contributed to writing the article, read and approved its submission.

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