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Ten Year Progression of *Musa* Breeding from 1987 to 1997: 1. Pollination Success and Seed Production (Fecundity) Patterns among Multiple Ploidy Crosses

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Authors' contributions

This work was carried out in collaboration among all authors. Authors WGF, SR, VD, OR, CJH, CHK, GF, PGC and APD were at various times involved in the conception, planning and execution of the research and in generating the data over the period 1987-1997. Author WV collated and performed the statistical analyses of the data and developed the manuscript and author TA provided access to the data.

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ABSTRACT

Aims: To create genetic variability, produce seeds and ultimately develop disease resistant and agronomically desirable hybrids with good organoleptic quality bunches.
Study Design: Multiple hand pollinations/ crosses in possible combinations of 2x, 3x and 4x as maternal/ seed and paternal/ pollen parents.

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[#]This Paper is Dedicated to the Memory of Dirk R. Vuylsteke, Musa Scientist who Died Tragically in an Air Crash on January 30, 2000 While on Active Duty. His Contributions to Plantain & Banana Agronomy, Taxonomy, Tissue Culture, Genetics and Breeding Remain Indelible.

Place and Duration of Study: International Institute of Tropical Agriculture High Rainfall Station (IITA), Onne, Rivers State, Nigeria. 1987 to 1997.
Methodology: Utilizing over 320 landraces, varieties, cultivars, clones/ genotypes and IITA accessions obtained from plantain and banana growing regions worldwide, scientists performed numerous hand pollinations/ crosses in possible combinations of 2x, 3x and 4x as maternal/ seed and paternal/ pollen parents every day.
Results: There were significant differences (*P*=.05) in number of seeds produced when diploids were used as maternal parents. Overall, 2x-2x produced 11times more seeds than the 2x-3x, and 54times more seeds than the 2x-4x. The 2x-2x crosses had double seed production maxima when crosses took place in June and July (8,300 seeds) and August and November (6,200 seeds) indicating a high level of fecundity. Only 2x-2x showed significantly positive correlation between seed production and pollination success (r = 0.617*). When triploids were maternal parents, there were significant differences (*P*=.05) in number of seeds produced. On average, 3x-2x produced 4times the number of seeds obtained from 3x-3x and 27times more than 3x-4x crosses. Seed production from triploids was 32times less than from diploids. The 3x-3x exhibited positively significant correlation between seed production and pollination success (r = 0.595*). With tetraploid maternal parents, there was significant difference (*P*=.05) in seed production when the male parent was diploid. High seed production (3,000-4,000) was achieved when pollination took place from June to October in the 4x-2x crosses. Seed production and pollination success in the 4x-2x were positively and highly significantly correlated (r = 0.865**).
Conclusion: In all crosses, diploid males produced the most seeds and pollination success increased as ploidy of maternal parent increased from diploid to tetraploid especially with diploid males.

Keywords: Genetic improvement; genome composition; ploidy level; ploidy cross types; maternal/ seed parent; paternal/pollen parent.

1. INTRODUCTION

One of the greatest constraints to genetic improvement of *Musa* is the limited production of viable seeds due to the complex ploidy, variable degrees of parthenocarpy / female sterility, and other factors affecting seed production in cultivated triploid and diploid plantains and bananas [1,2]. Breeding had been hampered not only when few viable seeds and seedlings are obtained, but also it requires 2 years in a seed-to-seed crop cycle [3]. Many authors documented that cross breeding of *Musa* spp.- the bananas and plantains is challenging because of the physiological and reproductive barriers of the plant itself, the differences between their sets of sub-genomes, and the plants being generally sterile, with seedless fruit [2-8]. Further reproductive barriers limit sexual recombination in banana and hinder plant improvement. Knowledge of the basic characteristics of dwarf and tall cultivars, desired ideotypes, results of genetic studies and combining ability tests, as well as specific objectives of the breeding programme are valuable guides in the selection of male and female parents along with resistance to biotic and abiotic stresses. Improvement of *Musa* is reported to be further complicated by parthenocarpy, reduced male fertility in some cultivars, low seed viability, irregular meiotic behaviour, long generation times, and diverse genomic configurations [9,7,10,11]. Earlier workers [9,12,8,] have outlined the major improvement and breeding objectives of *Musa* to entail hybridization and selection to evolve potential synthetic diploids and primary tetraploids to further generate triploid hybrids having good horticultural traits coupled with resistance or tolerance to pests and diseases such as the Sigatoka complex, multiple races of *Fusarium* wilt, bacterial wilt, bunchy top, nematodes, and weevils**.** Others are large uniform bunches/ a pendulous and large bunch with many hands and many long parthenocarpic fruits, superior fruit quality, early and high suckering ability/ regulated suckering behaviour, short stature/ dwarf type, and enhanced root systems that provide effective soil anchorage and efficient uptake of water and minerals (8,9). Other agronomic traits include early flowering and maturity, and an adequate flavour and texture, photosynthetic efficiency and rapid cycling are also important breeding objectives for increased yield (8). All of these required intimate knowledge of the ideotypes at the tetraploid, triploid and diploid levels, determination of parental effects, pollination time, seed production, progeny ploidy composition and location, in the production of true hybrid seeds in plantains and bananas. Although the foregoing list is not by any means exhaustive, a number of these breeding objectives still pose enormous challenges today because active and thriving collaborative program research on *Musa* breeding and improvement, as well as the support networks and structures, etc have been scaled back and declined in less than three decades. In 1987, plantains and bananas were included among the mandate crops of the International Institute of Tropical Agriculture (IITA) in Nigeria. A plantain and banana improvement program (PBIP) was subsequently created in 1991. The outcome of this genetic improvement program in plantain and bananas culminated among scientists at IITA developing and disseminating several high and stable yielding, disease and pest resistant hybrids with durable fruit quality and acceptability across Africa's plantain and banana growing regions, Latin America and India while several institutions have adopted IITA's different *Musa* breeding schemes [13-17]. This report attempts to bring into focus certain aspects of quantum of work through development of enormous cross combination breeding works comprising more than 600,000 controlled crosses from over 320 landraces, varieties, cultivars, clones/ genotypes and accessions conducted by scientists and field staff at the International Institute of Tropical Agriculture (IITA) High Rainfall Station, Onne, Rivers State, Nigeria, over the ten year period 1987-1997. Part of the efforts, to accelerate and expand knowledge of plantain improvement especially in the areas of controlled cross breeding and generation/ enhancement of genetic variability, production of seeds and transmission/ inheritance of desirable traits. Over the ten year period, numerous crosses were carried out by hand pollination of diploid (2x), triploid (3x) and tetraploid (4x) plantain/ banana maternal/ seed parents with pollen from 2x, 3x and 4x accessions of bananas and plantains in all possible but discriminatory/ selected combinations each day of every month of the year from January to December in order to determine and standardize different aspects viz (1) optimum time and conditions for pollination success and seed production for each ploidy cross type i.e. when pollination can lead to maximum seed production for each ploidy cross type; (2) the ploidy crosses that will provide the highest number of seeds (3) investigate whether all the ploidy crosses exhibit the same response pattern(s), (4) ascertain the ploidy nature of resulting progenies; (5) select parents that are able to transmit desirable traits and (6) establish which environmental factors critically affect or influence pollination success and seed production. Some of the environmental variables that affect these periodic phenological phenomena are known to include rainfall [18,19,20], temperature [21], solar radiation [22,23] and water stress [24]. Hence, this research study was conducted to create genetic variability, produce seeds and ultimately develop disease resistant and agronomically desirable hybrids with good organoleptic quality bunches.

2. MATERIALS AND METHODS

2.1 Description of Study Area

This study used data generated from research conducted at the High Rainfall Station of the International Institute of Tropical Agriculture (IITA) Onne, (4°51'N, 7° 03'E, 10m above sea level), Rivers State, Nigeria during 1987 to 1997. The rainfall pattern is monomodal, distributed over a 10- month period from February through December, with an annual average of 2400mm. Relative humidity remains high all year round with mean values of 78% in February, increasing to 89% in the months of July and September. The mean annual minimum and maximum temperatures are 25^0C and 27^0C, respectively, while solar radiation / sunshine lasts an average of 4hours daily [25]. The station is located in the rainforest characterized by an Ultisol/Acrisol (U.S. Department of Agriculture- USDA Taxonomy/ World Reference Base) derived from coastal sediments. The soil is a deep freely drained Typic Paleudult of loamy and siliceous iso-hyperthermic origin. The surface soils (0 - 15cm) are well drained and high in phosphorus 60 mg/kg, organic matter 1.85 %, but are low in total nitrogen 0.18% and also acidic with a pH of 4.6. Other nutrients are potassium 0.28meq/100g and magnesium 0.36meq/100g.

2.2 Planting and Plant Materials

Plants were grown at a spacing of 3m x 2m giving a population of 1,667plants/ha. Using a collection of 326 landraces, varieties, cultivars and clones/ genotypes, hybrids and IITA accessions of bananas and plantains (Tables 1,2,3,4) from banana and plantain growing regions around the world, crosses were carried out by hand pollination of diploid (2x), triploid (3x) and tetraploid (4x) plantain/ banana maternal/

seed parents with pollen from 2x, 3x and 4x of bananas and plantains in selected combinations everyday of each month of the year from January to December from 1987 to 1997 in order to produce viable seeds and also select parents that are able to transmit desirable traits.

Table 1. Landraces, Varieties, Cultivars, Clones/ Genotypes, Hybrids and IITA Accessions of Plantains and Bananas used as Maternal/ Seed Parents and or Paternal/ Pollen Parents with Genomic Composition, over period of 10years

S/N	Name	Genome* Composition	S/N	Name	Genome* Composition
1	Akondro Mainty	AA	47	Pisang tongat	AA
2	Bebek	AA	48	Pitu	AA
3	Beram	AA	49	Pitu No 1	AA
4	Calcutta 4	AA	50	Pu-te-la-bum	AA
5	Colatino Ouro	AA	51	Pu-Te-Wey	AA
6	Djum metek	AA	52	Racadag	AA
7	Djum tau	AA	53	Saing Todloh	AA
8	Galeo	AA	54	S.F. 214	AA
9	Guninchio	AA	55	S.F. 247	AA
10	Guyod	AA	56	S.F. 248	AA
11	Gwanhour	AA	57	S.F. 265	AA
12	Hawundu	AA	58	SH 3362	AA hybrid
13	Heva	AA	59	SN 2	AA
14	Higa	AA	60	Thong Det	AA
15	Inabaca	AA	61	Tjau Lagada	AA
16	Inarnibal	AA	62	Truncata	AA
17	Long Tannap	AA	63	Tuu Gia	AA
18	Long Tavoy	AA	64	Umbarim	AA
19	M. basjoo	AA	65	Undu Jamau	AA
20	M.Velutina	AA	66	Unknown Dibit	AA
21	Mambee Thu	AA	67	Uwati	AA
22	Manang	AA	68	Waigu	AA
23	Mojet	AA	69	Wariam	AA
24	Monget	AA	70	Wh-O-Gu	AA
25	Monjet	AA	71	Zebrina	AA
26	Morong Datu	AA	72	Zebrina GF	AA
27	Morong Princesa	AA	73	Zebrina GF062190	AA
28	*Musa* Pahang	AA	74	K46-1	AB
29	*Musa* A.Malaccensis	AA	75	Kamaramasenge	AB
30	*Musa* A.Selangor	AA	76	Unknown Mainz	AB
31	*Musa* A.Sp.Banksii	AA	77	*M. balbissiana*. MPL	BB
32	*Musa* acuminata	AA	78	*M. balbisiana*	BB
33	*Musa* siam	AA	79	Bakurura	AAA
34	Nba	AA	80	Biu ketip	AAA
35	No. 110	AA	81	Diploid basilian	AAA
36	Pa Musore	AA	82	Dwarf Cavendish	AAA
37	Pa Patthalong	AA	83	EMB 402	AAA
38	Paka	AA	84	EMB 403	AAA
39	Pamoti-On	AA	85	Giant Cavendish	AAA
40	Pisang buntal	AA	86	Hungtu	AAA
41	Pisang Jari Buaya	AA	87	Marauw	AAA
42	Pisang lilin	AA	88	Mbirabire	AAA
43	Pisang madu	AA	89	Mbwazirume	AAA
44	Pisang mas ayar	AA	90	Medja	AAA
45	Pisang Rotan	AA	91	Muga	AAA
46	Pisang songkla	AA	92	Mun	AAA

**[26,27,28,3,29]*

Table 2. Landraces, Varieties, Cultivars, Clones/ Genotypes, Hybrids and IITA Accessions of Plantains and Bananas used as Maternal/ Seed Parents and or Paternal/ Pollen Parents with Genomic Composition, over period of 10years

S/N	Name	Genome* Composition	S/N	Name	Genome* Composition
93	Nakitengwa	AAA	143	Mbi-Egome 1	AAB
94	Nyamwihogora	AAA	144	Mbouroukou	AAB
95	Ouro Mel	AAA	145	Moufoubila	AAB
96	Padri	AAA	146	Moutouka	AAB
97	Poyo	AAA	147	Muloulou	AAB
98	Robusta	AAA	148	Muracho	AAB
99	Rugondo	AAA	149	Nguma	AAB
100	Toowoolee	AAA	150	Niangafelo	AAB
101	Valery	AAA	151	Ntanga 4	AAB
102	75.19S	AAB	152	O.Ntanga 1	AAB
103	Abomienu	AAB	153	Obino L Ewai	AAB
104	Agbagba	AAB	154	Orishele	AAB
105	Akpakpak	AAB	155	Pisang Kelat	AAB
106	Amou	AAB	156	Pisang Raja	AAB
107	Apantu	AAB	157	Pisang Tondok	AAB
108	Apem Onniaba	AAB	158	Plantain No 17	AAB
109	Atali Kiogo	AAB	159	Plantain No. 2	AAB
110	Bind Immosendjo	AAB	160	Pome	AAB
111	Big Ebanga	AAB	161	Popoulou	AAB
112	Bise Egome	AAB	162	Purple plantain	AAB
113	Bobby Tannap	AAB	163	Rajapuri India	AAB
114	Bungaoisan	AAB	164	Red Plantain	AAB
115	Corne Plantain	AAB	165	Reversions	AAB
116	Curare	AAB	166	Tsambunu	AAB
117	Dare	AAB	167	Topala	AAB
118	Dare 111-30	AAB	168	Walungu	AAB
119	Didiede	AAB	169	Walungu 7	AAB
120	Dwarf French	AAB	170	Walungu 8	AAB
121	Eba Oboikpa	AAB	171	Wine Plantain	AAB
122	Eberedia	AAB	172	Bluggoe	ABB
123	Egjoga	AAB	173	Cardaba	ABB
124	Elat	AAB	174	Espermo	ABB
125	Essang	AAB	175	Fougamou	ABB
126	Igisahira Gisanzwe	AAA	176	Kinkala	ABB
127	Igitsiri	AAA	177	Pelipita	ABB
128	Imbongo	AAA	178	Simili Radjah	ABB
129	Ingarama	AAA	179	FHIA 2	AAAA
130	Ingumbay	AAA	180	FHIA 23	AAAA
131	Intokatoke	AAA	181	SH 3436	AAAA
132	French Reversion	AAB	182	SH 3436-9	AAAA
133	Gabon	AAB	183	SH 3640	AAAA
134	Gabon 3	AAB	184	FHIA 1	AAAB
135	Horn Plantain	AAB	185	FHIA 20	AAAB
136	Horse Plantain	AAB	186	FHIA 21	AAAB
137	Kar Ngou	AAB	187	FHIA 22	AAAB
138	Laknau	AAB	188	Oura de Mata	AAAB
139	M008	AAB	189	Platina	AAAB
140	Madre-D-Platanar	AAB	190	FHIA 3	AABB
141	Mbeta	AAB	191	Nzizi	AABB
142	Mbi Egome	AAB	192	Pisang Awak	AABB

** [26,27,28,3,29]*

Table 3. Landraces, Varieties, Cultivars, Clones/ Genotypes, Hybrids and IITA Accessions of Plantains and Bananas used as Maternal/ Seed Parents and or Paternal/ Pollen Parents with Genomic Composition, over period of 10years

S/N	Name	Genome† Composition	S/N	Name	Genome† Composition
193	Bobby Tannap & Calcutta 4 (4x)	NA	238	TMPx 15090-130	IITA accession
194	Obino Lewai & Calcutta 4(4x)	NA	239	TMPx 15108-1	IITA accession
195	Calcutta 4 & P. Lilin	NA	240	TMPx 15108-2	IITA accession
196	Calcutta 4, & P. Lilin & OP	NA	241	TMPx 15108-6	IITA accession
197	TM3x 1199-6	IITA accession	242	TMPx 1518-4	IITA accession
198	TM3x 14604-35	IITA accession	243	TMPx 1549-7	IITA accession
199	TM3x 15090-102	IITA accession	244	TMPx 15637-1	IITA accession
200	TM3x 15090-130	IITA accession	245	TMPx 1586-1	IITA accession
201	TM3x 15108-1	IITA accession	246	TMPx 1586-2	IITA accession
202	TM3x 15108-2	IITA accession	247	TMPx 1605-1	IITA accession
203	TM3x 15108-6	IITA accession	248	TMPx 1621-1	IITA accession
204	TMB2x 5105-1	IITA accession	249	TMPx 1657-12	IITA accession
205	TMB2x 8084-2	IITA accession	250	TMPx 1658-3	IITA accession
206	TMP2x 2829-62	IITA accession	251	TMPx 1658-4	IITA accession
207	TMPx 1055	IITA accession	252	TMPx 1659-9	IITA accession
208	TMPx 1112-1	IITA accession	253	TMPx 1668-7	IITA accession
209	TMPx 1112-1-857	IITA accession	254	TMPx 2143-4	IITA accession
210	TMPx 1112-1-858	IITA accession	255	TMPx 23835-1	IITA accession
211	TMPx 1112-1-859	IITA accession	256	TMPx 26200	IITA accession
212	TMPx 1112-1-860	IITA accession	257	TMPx 26217	IITA accession
213	TMPx 1112-1-861	IITA accession	258	TMPx 26231	IITA accession
214	TMPx 1112-1-862	IITA accession	259	TMPx 26240	IITA accession
215	TMPx 11669-1	IITA accession	260	TMPx 2625-20	IITA accession
216	TMPx 1206-2-1182	IITA accession	261	TMPX 26253	IITA accession
217	TMPx 1206-2-1543	IITA accession	262	TMPx 2625-5	IITA accession
218	TMPx 1206-2-1544	IITA accession	263	TMPx 26267	IITA accession
219	TMPx 1206-2-1545	IITA accession	264	TMPx 26285	IITA accession
220	TMPx 1206-2-1546	IITA accession	265	TMPx 26294	IITA accession
221	TMPx 1206-2-1547	IITA accession	266	TMPx 26298	IITA accession
222	TMPx 1206-2-1548	IITA accession	267	TMPx 26306	IITA accession
223	TMPx 129-3	IITA accession	268	TMPx 26312	IITA accession
224	TMPx 1297-3	IITA accession	269	TMPx 26313	IITA accession
225	TMPx 1297-3-849	IITA accession	270	TMPx 26338	IITA accession

S/N	Name	Genome† Composition	S/N	Name	Genome† Composition
226	TMPx 1297-3-851	IITA accession	271	TMPx 26352	IITA accession
227	TMPx 1297-3-852	IITA accession	272	TMPx 2637-49	IITA accession
228	TMPx 1297-3-853	IITA accession	273	TMPx 26392	IITA accession
229	TMPx 1378	IITA accession	274	TMPx 26421	IITA accession
230	TMPx 1418-2	IITA accession	275	TMPx 26466	IITA accession
231	TMPx 14280	IITA accession	276	TMPx 26505	IITA accession
232	TMPx 1448-1	IITA accession	277	TMPx 26525	IITA accession
233	TMPx 14563-1	IITA accession	278	TMPx 26535	IITA accession
234	TMPx 14604-35	IITA accession	279	TMPx 26613	IITA accession
235	TMPx 14604-37	IITA accession	280	TMPx 2776-2786-20	IITA accession
236	TMPx 1489-3	IITA accession	281	TMPx 2796-2848-5	IITA accession
237	TMPx 15090-102	IITA accession	282	TMPx 2796-3	IITA accession

† TM3X Series are secondary triploids [30]; TMPX Series are tetraploid plantains [14]; TMBX Series are tetraploid bananas [9]; TMP2X Series are diploid plantains [31]

Table 4. Landraces, Varieties, Cultivars, Clones/ Genotypes, Hybrids and IITA Accessions of Plantains and Bananas used as Maternal/ Seed Parents and or Paternal/ Pollen Parents with Genomic Composition, over period of 10years

S/N	Name	Genome† Composition	S/N	Name	Genome† Composition
283	TMPx 2796-5	IITA accession	308	TMPx 612-74 CAM	IITA accession
284	TMPx 2829-3019-16	IITA accession	309	TMPx 6562-1	IITA accession
285	TMPx 2829-62	IITA accession	310	TMPx 6930	IITA accession
286	TMPx 319-129	IITA accession	311	TMPx 6930-1	IITA accession
287	TMPx 319-132	IITA accession	312	TMPx 7002-1	IITA accession
288	TMPx 319-133	IITA accession	313	TMPx 7152	IITA accession
289	TMPx 3436-9	IITA accession	314	TMPx 7152-2	IITA accession
290	TMPx 3666-5	IITA accession	315	TMPx 7179-2	IITA accession
291	TMPx 4400-8	IITA accession	316	TMPx 7197-2	IITA accession
292	TMPx 447-80	IITA accession	317	TMPx 8084-3	IITA accession
293	TMPx 4479-1	IITA accession	318	TMP2x 2829-62 & TMB2x 5105-1	IITA accession
294	TMPx 4600-12	IITA accession	319	TMB2x 5105-1 & SH3362 & TMP2x 2829-62	IITA accession
295	TMPx 4600-15	IITA accession	320	TMPx 548-4 & SH 3362 (3x)	IITA accession
296	TMPx 4698-1	IITA accession	321	TMPx 4479-1 & SH 3362 (3x)	IITA accession
297	TMPx 4744-1	IITA accession	322	OP 13-153	NA
298	TMPx 5295-1	IITA accession	323	OP 13-1530621900	NA
299	TMPx 548-4	IITA accession	324	OP13-154	NA
300	TMPx 548-9	IITA accession	325	OP13-160	NA

S/N	Name	Genome† Composition	S/N	Name	Genome† Composition
301	TMPx 582-4	IITA accession	326	OP13-166	NA
302	TMPx 5511-2	IITA accession	327	OP13-169	NA
303	TMPx 565-16	IITA accession	328	OP13-27-161	NA
304	TMPx 5706-1	IITA accession			
305	TMPx 5860-1	IITA accession			
306	TMPx 597-2-490	IITA accession			
307	TMPx 612-74	IITA accession			

† TM3x series are secondary triploids [30]; TMPx series are tetraploid plantains [14]; TMBx series are tetraploid bananas [9]; TMP2x series are diploid plantains [31]

2.3 Hand Pollination

The inflorescence of the females and males were protected by transparent plastic bags before anthesis of either of the flowers. At anthesis pollen from the anthers of the males was dusted on the stigmas of the females and this was done between 0700 and 1030hours. Thus the following crosses were carried out with diploid plantains/ bananas as maternal /seed parents:- 2x-2x, 2x-3x, 2x-4x; triploid plantains/ bananas as maternal/ seed parents:- 3x-2x, 3x-3x, 3x-4x; and tetraploid plantains/ bananas as maternal/ seed parents: - 4x-2x, 4x-3x, 4x-4x.

2.4 Bunch Harvest and Extraction of Seeds

At maturity the fruit bunches of the maternal/ seed parents were harvested and ripened. Well developed seeds from each maternal/ seed parent were extracted, washed and air-dried. and the total number of such seeds was counted.

2.5 Data Collection

The data on pollination success and seed production were aggregated over the period, averaged and standard errors were computed. The total number of crosses that produced seeds (pollination success) and number of seeds (seed production - fecundity) per cross type were calculated (Equation 1) and expressed as percentages to establish pollination success on a monthly basis before presentation in graphical form.

Pollination success was calculated as shown below (17)

$$\text{Pollination success} = \frac{\text{Number of pollinated bunches with seed}}{\text{Total number of pollinated bunches}} \text{X } 100 \qquad (1)$$

Total seed yield for each seed parent was also calculated. Seed production and pollination success values were plotted monthly for the period.

2.6 Statistical Analysis

All data were analyzed for significant differences using analysis of variance (ANOVA) in a randomized complete block design (RCB) using the GLM procedure of Statistical Analyses Software (SAS) version 9.1 [32] and any effects found to be significant were tested at a significance level of 5% while means were compared using LSD at (*P* = .05). Correlation analyses [33] were carried out to determine if there was a linear relationship between seed production and pollination success among the various ploidy cross types at significance levels of 5% and 1%.

3. RESULTS AND DISCUSSION

3.1 Effect of Diploid Plantain/ Banana as Maternal/ Seed Parents

There were significant differences (*P*=.05) in the number of seeds produced by the various ploidy cross types when a diploid plantain/ banana was used as maternal/ seed parent (Fig. 1A). Overall, the 2x-2x cross types produced 11times (1098%) more seeds than the 2x-3x cross types and 54times (5402%) more seeds than the 2x-4x cross types. On average, over the 10 years, the 2x-2x cross types produced the highest number of seeds and this occurred when pollination/ crosses took place in June and July, with over 8,300 seeds indicating a high level of fecundity – an ability to produce seeds in large numbers. Next was when pollination took place in November and August with over 6,200 seeds. Thus, the 2x-2x cross types exhibited a double maxima (double peak) of high seed production, even though pollination success rates were different when pollination took place in the drier times/ season of the year. There was a significant difference in pollination success (*P*=.05) between the 2x ploidy cross types. Whereas pollination success of the 2x-2x cross types and 2x-3x cross types were not significantly different from each other, both were significantly different from the pollination success of the 2x-4x cross types. Pollination success of the diploid ploidy cross types did not differ significantly from each other irrespective of month of pollination. Pollination success in June, July and August, November for the 2x-2x crosses when highest seed production was achieved were just 32%, 34% and 33% and 18% respectively (Fig.1B).

Incidentally the highest pollination success of 37% for this 2x-2x cross type was in September when seed production was just a third of seed production in June and July indicating perhaps that seed production and pollination success did not share a strong relationship, with the possibility of other factors playing a modifying role. In fact only the 2x-2x cross type showed significant and positive correlation between seed production and pollination success (r = 0.617*), whereas other cross types 2x-3x (r = 0.361) and 2x-4x (r = 0.025) were not significant. In every month of the year, 2x-2x crosses produced more seeds than the 2x-3x and the 2x-4x crosses except in April and May when the 2x-3x crosses produced more seeds. Peak production of seeds in the 2x-3x crosses occurred in May (1,297) and April (1,260) when pollination success rates were 13% and 37% respectively. The 2x-4x crosses produced the lowest number of seeds in each of the months. For this 2x-4x cross type, highest seed production of 292 seeds was in December (2% pollination success), 163 seeds in August (10% pollination success) and 112 seeds in May (21% pollination success) indicating again that seed production and pollination success in *Musa* do not share a strong relationship. The 2x-2x and the 2x–4x crosses produced predominantly diploid progenies while the 2x-3x crosses had predominantly tetraploids. With diploid plantain as maternal parent, none of the ploidy crosses achieved up to 40% pollination success even though highest seed production occurred with this ploidy cross. Average pollination success was 24% among the 2x-2x crosses, 17% among the 2x-3x crosses and 10% among the 2x-4x crosses.

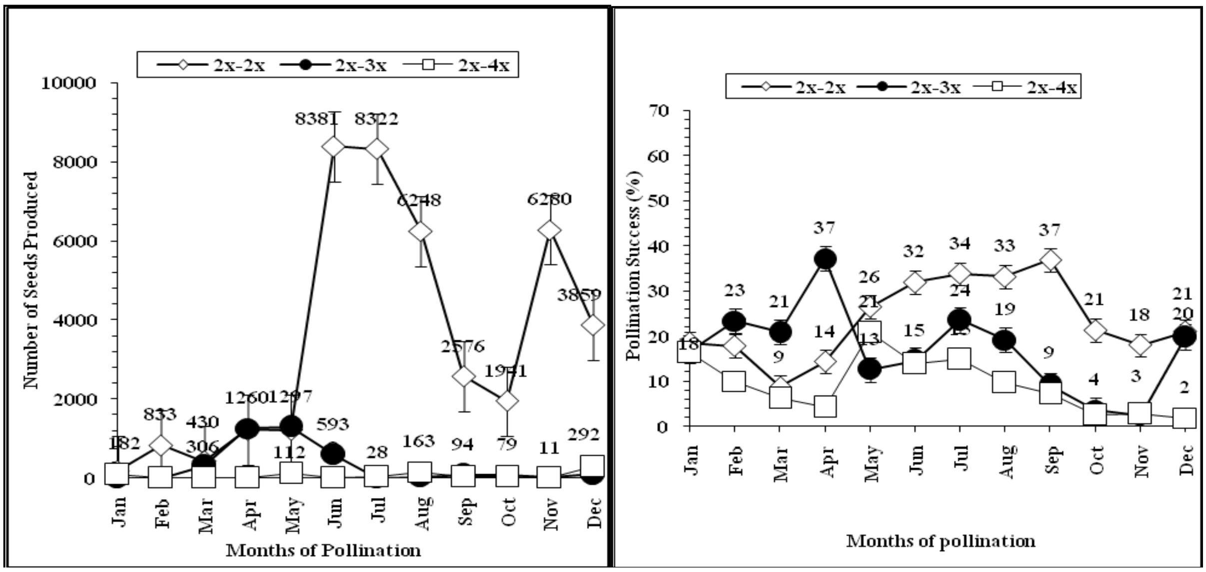
3.2 Effect of Triploid Plantain/ Banana as Maternal/ Seed Parents

There were significant differences (*P*=.05) in the number of seeds produced by the various ploidy cross types when a triploid plantain/ banana was the maternal/ seed parent. On the average, the 3x-2x cross types produced 4times (400%) the number of seeds produced by the 3x-3x crosses and 27times (2700%) more than the 3x-4x crosses (Fig. 2A). Generally, when triploids were maternal/ seed parents, number of seeds produced on average was quite low compared to when diploids were maternal/ seed parents.

The 3x-2x crosses had significantly higher pollination success than both 3x-3x and 3x-4x cross types (Fig. 2B). The 3x-3x crosses also had significantly higher pollination success than the 3x-4x crosses. Generally, pollination success also differed significantly with the month of pollination when triploids were used as maternal/ seed parents. For the months of February, March, April and June pollination success did not differ significantly from each other. July and December also did not differ significantly from each other neither did August and November. On the whole, pollination success differed significantly from each other in 7 instances of the 12months with September averaging the highest across the cross types and May the lowest. The 3x-2x crosses in April and July had the highest number of seeds (258 and 234 seeds, respectively) corresponding to 30% and 40% pollination success respectively. Pollination success was highest in the 3x-2x crosses in September reaching 45% with only 141 seeds produced, again indicating that seed production and pollination success may not have a very strong relationship. The 3x–3x crosses resulted in an average of 119 seeds; the highest when pollination was carried out in November with 19% pollination success. Pollination success for 3x-3x was highest (25%) when pollination was done in October but produced only 68 seeds. The 3x–4x crosses done in October had their highest number of seeds with an average of 34 seeds corresponding to an 18% pollination success, whereas the highest pollination success of this cross type-22% in September produced just 11 seeds. The implication is that the triploids have low fecundity as they are unable to produce seeds in large numbers and do not make for good maternal/ seed parents irrespective of the ploidy of the male/ pollen parent. Whereas the 3x-2x (r = 0.133) and the 3x-4x (r = 0.527) showed weak correlation between seed production and pollination success, the 3x-3x had a positive and significant correlation between seed production and pollination success (r = 0.595*). On average pollination success of the 3x-2x crosses was two and a half times more than the 3x-3x crosses and five and a half times more than the 3x-4x crosses.

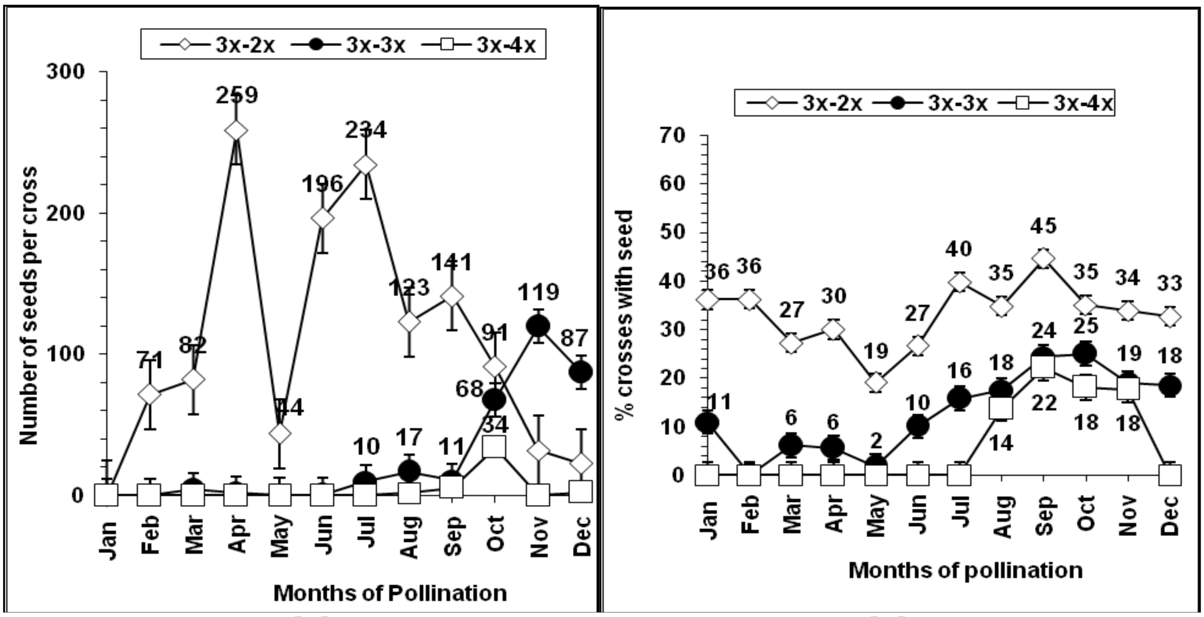
3.3 Effect of Tetraploid Plantain/ Banana as Maternal/ Seed Parents

When tetraploids were used as maternal/ seed parents, there were significant differences (*P*=.05) in the seed production obtained depending on the ploidy of the male/ pollen parent. High seed production i.e. high fecundity was achieved when pollination took place from June to October in the 4x-2x crosses ranging from approximately 3,000 to 4,000 seeds. The other cross types, 4x-3x and 4x–4x did not achieve a seed production of up to 250 seeds irrespective of month of pollination (Fig. 3A). In those five months, seed production in the 4x-2x crosses was consistently averaging more than 3,000 seeds with a maximum in October of 4,233 seeds. Pollination success differed significantly with cross types. The 4x-2x crosses differed significantly from the 4x-3x and the 4x-4x which did not differ significantly from each other (Fig. 3B). The 4x–2x crosses also achieved at least a 45% pollination success from May to October with a peak of 68% in July. Pollination success remained below 40% irrespective of month of pollination in the 4x-3x and 4x-4x cross types. In fact, the 4x–3x crosses achieved their highest pollination success of 36% in February while the 4x–4x crosses had their highest pollination success of 33% in December. There was a highly significant and positive correlation between seed production and pollination success in the 4x-2x cross type (r = 0.865**), whereas other cross types 4x-3x showed little correlation (r = 0.054) and 4x-4x even negative but not significant correlation (r = -0.556).



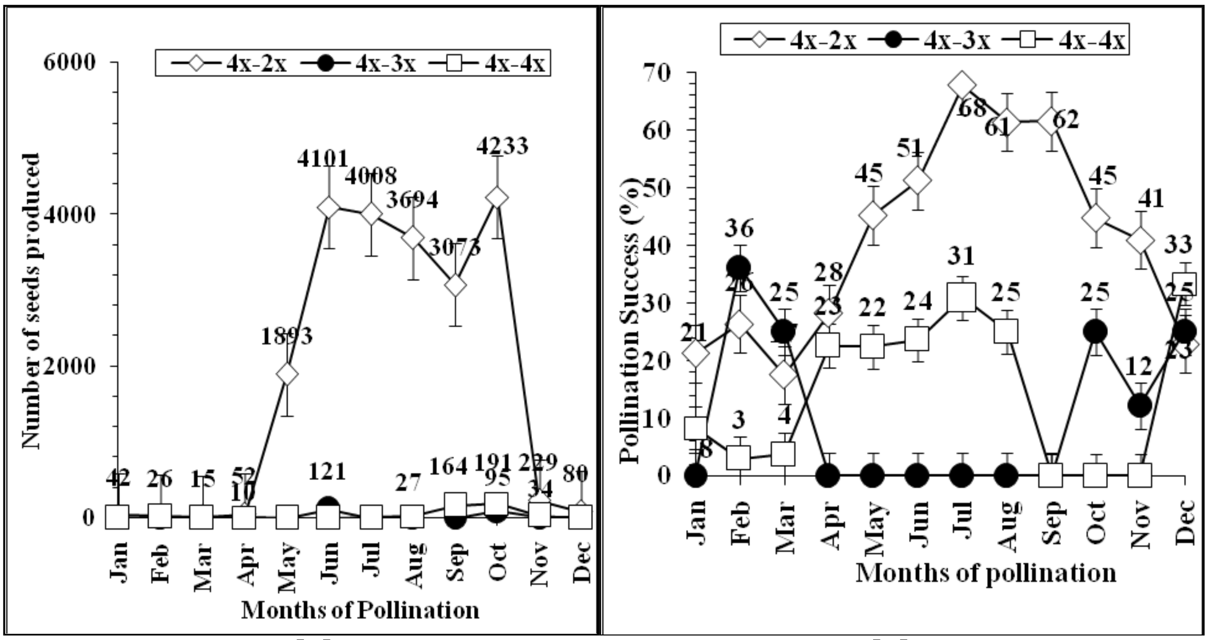
[A] [B]

Fig. 1. [A] Seed Production (Fecundity) and [B] Pollination Success patterns in diploid, maternal/ seed parents of plantain/ banana from monthly crosses with pollen from 2x, 3x and 4x banana and plantain paternal/ male parents averaged over 10 years (1987-1997) at IITA, Onne, Rivers State, Nigeria



[A] [B]

Fig. 2. [A] Seed Production (Fecundity) and [B] Pollination Success patterns in triploid, maternal/ seed parents of plantain/ banana from monthly crosses with pollen from 2x, 3x and 4x banana and plantain paternal/ male parents averaged over 10 years (1987-1997) at IITA, Onne, Rivers State, Nigeria



[A] **[B]**

Fig. 3. [A] Seed Production (Fecundity) and [B] Pollination Success patterns in tetraploid, maternal/ seed parents of plantain/ banana from monthly crosses with pollen from 2x, 3x and 4x banana and plantain paternal/ male parents averaged over 10 years (1987-1997) at IITA, Onne, Rivers State, Nigeria

Generally, comparing all nine ploidy crosses, the 2x-2x cross produced the highest number of seeds and the optimal times for obtaining maximum seed production was when crosses were carried out in June and July, with lower peaks in August and November. The high seed production in the 2x-2x may be indicative of both female and male fertility in the diploid background. High seed yields were not observed when the pollen source was 3x or 4x. The 4x-2x crosses also showed high fecundity though only achieving approximately half of the seed production attained in the 2x–2x crosses but having almost double the level of pollination success. However, the crosses involving triploids as maternal/ seed parents had the lowest number of seeds, producing less than an average of 300seeds maximum irrespective of when pollination took place indicating that the triploids have very low fecundity and are not suitable as maternal/ seed parents [8] which complicates breeding efforts. For seed stock multiplication, 2x-2x and 4x–2x appear to be the most viable options. Pollination success was generally low and in some instances was zero as reported [17] but this increased as the ploidy level of the maternal parent increased from diploid to tetraploid especially when the male parent was a diploid. Previous studies in cotton (*Gossypium hirsutum* L.) revealed that when species with more chromosomes were used as the maternal parent, the cross was more easily made, and viable seeds were produced. When the species with fewer chromosomes was used as the female, no viable seeds were obtained [34]. Similar phenomena were observed in tomato (*Lycopersicon esculentum,* Miller) [35] and false pakchoi (*Brassica parachinensis* Bailey) [36]. The highest pollination success (68%) was achieved with the 4x-2x crosses in July with relatively high seed production. On the whole, month of pollination did not significantly affect pollination success except when triploids were used as maternal parents. Similar findings were reported earlier [37]. However, some researchers in their findings [17] reported no significant differences in pollination success based on month of pollination. More studies are clearly needed to find out why triploid maternal parents exhibit peculiarities different from the other ploidy cross types. Sterility in plantains and triploid bananas has been associated with meiotic irregularities and uneven number of chromosomes, as well as to environmental factors and to influences of individual genotypes [13,38]. The highest seed production for 2x-2x crosses was when hand pollinations were performed in June, July, August and November and for 4x-2x crosses when hand pollinations were done from June to October; while pollination of 3x with 2x pollen produced highest number of seeds when crosses were done in April and July a period with high temperature, high solar radiation and low relative humidity [37] suggesting that seed yield is influenced by time of pollination, environmental conditions, and genetic variation in female fertility. Variations in seed production among cross types suggest that seed production may be affected by climatic factors and this will be discussed in a subsequent paper.

4. CONCLUSION

High seed production in *Musa* can be achieved by using diploids and tetraploids as female or maternal/ seed parents with diploid male or paternal/ pollen parents with pollination successes ranging from 32% to 68% when crosses are carried out from June to August/ September (2x-2x; 4x-2x) as well as in October (4x-2x) and November (2x-2x).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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