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Seasonal Variation of Apparent Male Fertility and 2n Pollen Production in Plantain and Banana

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Abstract. **Current efforts to produce improved genotypes of plantain (*Musa* spp. AAB genomic group) and banana (*Musa* spp. AAA genomic group) depend on identifying triploid, female-fertile clones and crossing them with male-fertile, diploid wild or cultivated accessions. Apparent male fertility, as measured by pollen stainability, and production of 2n pollen (gamete with the sporophytic chromosome number) were examined over a period of 1 year (Oct. 1992 to Oct. 1993) in 'Calcutta 4' (wild banana), 'Galeo', and 'Pisang lilin' (cultivated bananas), and in TMP2x 1297-3 (plantain–banana hybrid), which are the most popular diploid parents in the breeding program of the International Institute of Tropical Agriculture. Differences in pollen stainability were found among these clones. However, a seasonal variation in pollen stainability was observed in all clones except 'Calcutta 4'. Solar radiation was positively associated ($P < 0.05$) with pollen stainability. Pollen stainability in the diploid banana parents was compared with seed set after triploid-diploid crosses between plantains and bananas. There was a clear difference in the capacity of male parents to fertilize, but seed set was not significantly correlated with pollen stainability ($r = 0.246, P = 0.358$). Although the seasonal maximum seed set coincided with the time of maximum pollen stainability, variation in seed set seems to be due mainly to seasonal variation in female fertility. Of all clones examined, only 'Pisang lilin' produced 2n pollen throughout the year. Seasonal variation in 2n pollen production was highly correlated ($P < 0.05$) with solar radiation, temperature, total pan evaporation, rainfall, and minimum relative humidity. The identification of male-fertile 2n pollen-producing diploid accessions, and of the best time of the year to maximize fertility and 2n pollen production, will allow the synthesis of polyploid *Musa* hybrids through sexual polyploidization.**

Bananas (*Musa* spp. AAA genomic group), cooking bananas (*Musa* spp. ABB genomic group), and plantains (*Musa* spp. AAB genomic group) are important food crops in tropical countries (Robinson, 1996). Pests and diseases are the major constraints to banana and plantain production worldwide (Gowen, 1995; Jeger et al., 1995). Host plant resistance is the most ecologically sustainable plant protection strategy to control *Musa* pests and diseases and enhance plantain and banana production. Recently, tetraploid hybrids resistant to major biotic constraints (e.g., sigatoka leaf spots and Panama disease) have been selected through conventional cross-breeding (Ortiz et al., 1995).

Gametes with the sporophytic chromosome number are known as 2n gametes (Mendiburu and Peloquin, 1976). Such gametes are the result of a modified gametogenesis under the genetic control of meiotic mutants, which may be affected by the environment (Koduru and Rao, 1981; Veilleux, 1985). In *Musa* spp. L., 2n eggs opened the path for the genetic improvement of bananas (Rowe, 1984) and plantains (Vuylsteke et al., 1997). Furthermore, introgression of desirable alleles from diploid species to polyploids could be achieved either through unilateral (2n x n or n x 2n) or bilateral (2n x 2n) sexual polyploidization in *Musa*.

Production of hybrid seed depends on the fertility of both parents, which may be influenced by the environment (Ortiz and Vuylsteke, 1995). Hence, the objectives of this research were to determine the seasonal variation in male fertility (as measured by pollen stainability) and 2n pollen production in widely used diploid male parents in *Musa* breeding, and to identify the climatic factors affecting both pollen stainability and 2n pollen production.

Materials and Methods

Pollen analysis. Pollen samples were collected daily from Oct. 1992 to Oct. 1993 in flowering plants of the following diploid ($2n = 2x = 22$) banana (*Musa acuminata* Colla.) accessions from Asia and the Pacific: 'Calcutta 4' (wild banana), and 'Galeo' and 'Pisang lilin' (cultivated bananas). Also, pollen was collected from TMP2x 1297-2, a plantain-derived diploid hybrid developed at IITA from a cross between a triploid ($2n = 3x = 33$) plantain (AAB genomic group) and 'Calcutta 4'. This research was carried out at the International Institute of Tropical Agriculture (IITA) High Rainfall Station in southeastern Nigeria (Ortiz et al., 1997). This location, Onne, has a swamp vegetation and is in the secondary center of plantain diversification in the humid lowland rainforest of West Africa. A monthly summary of weather data at Onne from Oct. 1992 to Oct. 1993 is shown in Table 1.

At anthesis, male flowers were excised from the male bud between 7:30 and 10:30 AM and pollen grains dislodged from the stamen, spread on a microscope slide and stained with acetocarmine glycerol jelly (Marks, 1954). Two hundred pollen grains from two random samples were observed under a Leitz Diaplan binocular light microscope (×400 magnification). Only completely rounded and deeply stained grains were considered as viable pollen in this experiment. The percentage of pollen stainability was calculated to determine the level of apparent male fertility of the diploid accessions (Dumpe and Ortiz, 1996). Stainability with acetocarmine glycerol is an established method to determine pollen viability for estimating the level of male fertility of *Musa* clones (Dessauw, 1988).

The diameter of viable grains was measured with the aid of a graduated eyepiece for an accurate determination of pollen sizes. Giant pollen grains having diameters ≥160 μ were classified as 2n pollen, since 2n pollen normally has 1.25 times the length of haploid or n pollen (Darlington, 1937), which never exceeds 128 μ in non-2n pollen producers of *Musa* (Ortiz, 1997). The frequency of 2n pollen was calculated as the ratio [number of giant pollen grains (i.e., those >160 μ)/total number of viable pollen observed in the corresponding sample].

Crossability. Two triploid French plantain cultivars, 'Mbi Egome 1' and 'Obino l' Ewai' (from Nigeria), were selected for controlled triploid-diploid crosses with the three banana diploids. Pollen of the male parents was collected around 7:30 AM from male flowers, which were previously covered with cotton cloth bags to prevent pollen contamination with other sources due to animal activity. Likewise, inflorescences of the female parents were bagged from shooting until the last female flower was pollinated to avoid natural crossing with an unidentified pollen source. Artificial hand pollinations were done between 7:30 and 10:30 AM in exposed female flowers by rubbing a cluster of male flowers onto the female flowers. Pollinated bunches were tagged, indicating parents and date of initial pollination, and harvested ≈90 d from flowering. Harvested bunches were stored for 4 d in ripening rooms containing acetylene. Seeds were extracted manually after squash-

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Table 1. Monthly summary of weather data at Onne, Nigeria, from Oct. 1992 to Oct. 1993.

Month	Total rainfall (mm)	Total evaporation (mm)	Mean wind speed (km·h⁻¹)	Solar radiation[z] (MJ·m⁻²·day⁻¹)	Temp (°C) Min	Max.	Mean	Relative humidity (%) Min.	Max.	Mean	No. rainy days[y]
October	299.0	83.2	3.1	12.0	22.5	28.9	25.7	72	97	84	21
November	98.9	90.5	2.3	13.8	21.5	30.7	26.1	60	96	75	12
December	17.9	104.0	2.2	15.1	21.5	32.2	26.9	48	97	73	2
January	1.5	126.8	2.9	14.2	20.5	32.1	26.3	42	88	67	6
February	41.7	119.3	3.7	16.0	22.2	33.4	27.8	49	96	73	8
March	250.8	139.4	3.5	15.3	22.2	32.1	27.1	55	96	74	11
April	130.5	111.1	3.1	15.7	22.6	31.5	27.0	62	97	79	14
May	212.1	116.4	3.1	14.8	22.4	31.1	26.8	66	97	77	17
June	320.0	84.5	2.8	11.3	22.0	29.3	25.6	71	97	81	22
July	512.5	52.1	2.8	9.0	22.5	28.0	24.9	78	98	74	25
August	438.2	61.6	2.9	9.2	22.8	27.9	25.3	76	96	80	24
September	248.1	75.7	2.8	12.0	22.9	29.3	26.1	68	96	79	24
October	262.8	97.0	2.7	13.4	22.3	30.4	26.3	64	96	77	14

[z]MJ·m⁻²·day⁻¹ = (Gm-cal·cm⁻²·day⁻¹)/23.923.
[y]A rainy day was recorded when rainfall ≥ 0.2 mm.

ing the ripened peeled fruits with a mechanical pressing machine, and were counted from each independent pollinated bunch to determine seed set per bunch.

Regression analyses were performed to determine linear relationships between weather data (independent variables) and pollen stainability and 2n pollen production (dependent variable). Correlation analysis was used to determine the association between pollen stainability and seed set after controlled triploid-diploid crosses.

Results and Discussion

The success of *Musa* breeding depends on the identification of triploid seed-fertile landraces for further crosses with wild or cultivated diploid bananas (Vuylsteke et al., 1997), improved diploid bananas (Rowe and Rosales, 1996), or plantain-derived hybrids (Vuylsteke and Ortiz, 1995). The local landrace normally provides genes for local adaptation and fruit quality, while the diploid male parents are the source of resistance to a specific pest or disease. The success of *Musa* cross-breeding requires the production of hybrid seed from interspecific and/or interploidy crosses.

Hybrid seed production is significantly influenced by the seed fertility of the female parent or season of pollination (Ortiz and Vuylsteke, 1995). Likewise, environmental effects influence pollen stainability as observed in this experiment. 'Calcutta 4', the wild banana, had very high levels of pollen stainability throughout the year, whereas pollen stainability varied with season in the other diploids (Table 2). Maximum pollen stainability in 'Galeo', 'Pisang lilin', and TMPx 1297-3 was obtained from January to April. Changes in total pan evaporation, solar radiation, maximum and mean temperature, minimum relative humidity, and rainfall were significantly ($P < 0.05$) associated with the seasonal variation for pollen stainability (Table 3). High solar radiation, high temperature, and high total pan evaporation significantly ($P < 0.05$) enhanced pollen stainability, whereas high rainfall and high minimum relative humidity significantly ($P < 0.05$) reduced it.

The highest number of hybrid seeds in plantain–banana crosses were always obtained with the most fertile male parent, 'Calcutta 4' (Table 4). In contrast, the cultivated diploid bananas 'Pisang lilin' and 'Galeo', which have lower pollen production than 'Calcutta 4' (Dumpe and Ortiz, 1996), had low seed set in crosses with the triploid plantains (Table 4). The association between pollen stainability and seed set was nonsignificant ($r = 0.246$, $P = 0.358$). Hence, seed set in triploid-diploid crosses was not significantly affected by the apparent male fertility, as measured by pollen stainability. The low level of pollen stainability of 'Pisang lilin' (syn. 'Pisang lidi') could result from its structural hybridity due to a chromosomal interchange (Hutchinson, 1966). Similarly, 'Galeo' appears to be a reciprocal translocation heterozygote (Faure et al., 1993).

'Pisang lilin' had 2n pollen, production of which varied throughout the period of this study. This observation suggested that environmental factors affected 2n pollen production in this cultivated diploid. Environments characterized by high solar radiation apparently enhance the production of 2n pollen. The significant ($P < 0.05$) linear relationship between solar radiation (MJ·m⁻²·day⁻¹) and 2n pollen production in the diploid banana cultivar Pisang lilin was: 2n pollen (%) = –30.489 + 4.212 solar radiation ($R^2 = 0.715$). The production of 2n pollen was also positively associated with pan evaporation ($r = 0.790$, $P = 0.001$), and temperature ($r = 0.809$, $P = 0.001$), and negatively associated with rainfall ($r = -0.625$, $P = 0.021$) and minimum relative humidity ($r = -0.649$, $P = 0.015$). Similarly, high solar radiation along with high temperature and low relative humidity enhance the production of 2n eggs in *Musa* (Ortiz and Vuylsteke, 1995).

Polyploid hybrids are the most desirable genotypes in *Musa* breeding because of their high yield (Vuylsteke et al., 1997). Crosses for the production of such *Musa* hybrids should be made in environments that maximize fertility and 2n pollen production, e.g., months with high solar radiation. This may allow the synthesis of many polyploid *Musa* hybrids through sexual polyploidization.

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Table 2. Mean monthly pollen stainability (%) in diploid *Musa* accessions at Onne, Nigeria (1992–93).

Month	Calcutta 4	Galeo	Pisang lilin	TMP2x 1297-3
October	98	51	44	---[z]
November	99	98	66	78
December	99	96	77	92
January	99	98	89	94
February	99	97	92	96
March	99	97	92	94
April	98	97	93	96
May	98	98	93	89
June	99	---	57	54
July	100	---	44	50
August	100	78	42	---
September	99	81	44	58
October	99	79	50	57

[z]No plants of this accession flowered in this month.

Table 3. Correlation coefficients (*r*) between weather variables and pollen stainability of diploid accessions (Onne, Nigeria, Oct. 1992 to Oct. 1993).

Statistics	Total rainfall	Total evaporation	Mean wind speed	Solar radiation	Temp Min.	Temp Max.	Temp Mean	Relative humidity Min.	Relative humidity Max.	Relative humidity Mean	No. rainy days
					Calcutta 4						
r	0.437	−0.506	−0.216	−0.614	−0.011	−0.379	−0.482	0.190	−0.057	−0.085	0.215
P	0.133	0.075	0.477	0.024	0.972	0.199	0.093	0.532	0.853	0.343	0.480
					Galeo						
r	−0.636	0.634	−0.003	0.674	−0.447	0.740	0.646	−0.657	−0.233	−0.744	−0.634
P	0.033	0.034	0.994	0.021	0.165	0.008	0.029	0.026	0.489	0.008	0.034
					Pisang lilin						
r	−0.708	0.897	0.398	0.868	−0.419	0.888	0.832	−0.742	−0.296	−0.542	−0.725
P	0.006	<0.001	0.175	<0.001	0.152	<0.001	<0.001	0.003	0.324	0.053	0.004
					TMP2x 1297-3						
r	−0.779	0.856	0.366	0.904	−0.369	0.912	0.831	−0.790	−0.321	−0.476	−0.786
P	0.004	0.001	0.265	<0.001	0.261	<0.001	0.001	0.003	0.332	0.135	0.003

Table 4. Seed set (seeds per bunch) in triploid-diploid crosses between unrelated plantains and bananas for 2 months of pollination in the rainy (R, Oct. 1992) and dry (D, Jan. 1993) seasons.

Diploid banana (male parent)	Triploid plantain (female parent) Obino l' Ewai R	D	Mbi Egome 1 R	D
Calcutta 4	7.4	5.8	12.9	19.3
Galeo	0.0	1.2	6.0	14.5
Pisang lilin	2.8	0.0	2.0	0.0

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