Nig J. Biotech. Vol. 27 (2014) 21-27
ISSN: 0189 17131
Available online at http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org.

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Pollen diversity, viability and floral structure of some *Musa* genotypes

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(Received 11:1:2013; Accepted 10:7:14)

Abstract
This experiment was designed to study the floral structure, pollen morphology and the potential pollen viability of five *Musa* genotypes obtained from the *Musa* field germplasm bank at the Faculty of Agriculture & Natural Resources Management farm, Ebonyi State University, Abakaliki. Palynological investigation was carried out in the Applied Biology Laboratory of the same University. Results indicated significant differences (P < 0.001) in style and anther length among the genotypes. 'Agbagba' (a triploid landrace genotype) had the longest style (4.31±0.046cm) and anther (4.81±0.028 cm) compared with the other genotypes. Palynologically, results from pollen morphology studies indicated significant (P < 0.001) differences among the genotypes for pollen sizes and apertural types. Three different types of pollen were encountered viz, big, moderate and small pollens with corresponding big, moderate and small apertures and pores. The highest value for pore diameter was recorded for FHIA 25 (33.3±1.9 μm). There were apertural differences among the *Musa* genotypes studied. 'Agbagba' pollen grains were polyporate while others were polycolporate. PITA 14 pollen grains were the most deeply stained and thus most viable compared to the other four genotypes. This genotype could therefore serve as potential male parents in crosses.

Key words: *Musa* species, pollen stainability, viability, apertural types, parental crosses
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Introduction

Plantains and bananas (*Musa* spp) are giant perennial grasses that are cultivated in the tropical and sub-tropical regions of the world where they serve as staple and cash crops. The cultivated varieties are predominantly triploids (2n =3x =33) and develop fruits by vegetative parthenocarpy. Few species are diploids or tetraploids. Plantain and banana breeding has attempted to mimic the evolutionary development of the *Musa* species complex (Rowe and Rosales, 1996; Ortiz, 1997) from inter-specific hybridization and polyploidization involving *Musa acuminata* Colla and *M. balbisiana* Colla. Thus, female fertile triploid landraces of plantain (AAB genomes) are crossed to diploid accessions of *M. acuminata* or *M. balbisiana* that are resistant to black Sigatoka. However, genetic improvement within the species has been slow due to limited understanding of the genetic organisation and meiotic behaviour of the species (Ortiz and Vuylsteke, 1996). This is related to sterility due to triploidy, variation in genome size and structure both across and within generations.

Flowering, pollination and fertilization are critical factors that determine fruit set in the plant kingdom. Pollination is largely determined by floral variations. An understanding of the floral characteristics of different *Musa* varieties may be indicative of their behaviour during pollination and fertilization. Several barriers to fertilization, including variability in pollen production, poor or malfunctioning pollen grains, may occur among genotypes. Other factors include both internal and external factors that limit pollen production, viability and germination rate in different crops. For example, low temperature during flowering reduces pollen germination, inhibiting pollen tube growth thereby resulting in poor fertilization and yield (AGP, 2003).

Pollen grains are structures that house the male gametophytes generation of seed plants and are the vehicles through which the microsporangium is carried to the female gamete. The pollen grain has a very complex structure that may be reflective of specific species' functional adaptations. The ability of pollen grains to effect fertilization and hence fruit set is dependent on their viability. Pollen viability is generally considered to indicate the ability of the pollen grain to perform its function of delivering the sperm cells to the embryo sac following compatible pollination (Shivanna *et al.,* 1991). Apparently, male fertility and viability are measured by pollen stainability and differences in pollen viability among genotypes are largely of genetic origin. Generation of improved genotypes of plantains (*Musa spp* AAB genomic group) and bananas (*Musa spp*, AAA genomic group) depend on identifying triploid female fertile clones and crossing them with male fertile, diploid wild or cultivated accessions.

Broad variation in pollen viability exists among clones within *Musa* species. Generally, diploid *Musa* species produce more viable pollen than tetraploids which, in turn produce more viable pollen than triploids. A mathematical approach to the evaluation of pollen diameter was used (Veronessi *et al*., 1988; Tondini *et al*., 1993) in which the diameter of pollen of tetraploids was comparable to 2n pollen diameter of diploids and bigger than n pollen of diploids. Fertility of triploid *Musa* genotypes at EBSU field germplasm may be consequent upon factors identified above. The establishment of a *Musa* breeding scheme in the University would be dependent upon an understanding of the floral characteristics, pollen features and viability of the different genotypes in the field germplasm. The objectives of this study includes the determination of the floral structures of five *Musa* genotypes from Ebonyi State University *Musa* Field Germplasm, evaluation of the pollen features of these genotypes, and the estimation of the pollen variability and viability of the *Musa* genotypes.

Materials and Method

Experimental Site and Genetic Material: This study was carried out in the laboratory of the Department of Applied Biology, Faculty of Biological Sciences, Ebonyi State Universality, Abakaliki. The Genetic materials were made up of FHIA 25, Lagos banana (LBN), PITA 14, PITA 17 and 'Agbagba' (a landrace genotype). The polleniferous buds from these genotypes were collected from the Field Germplasm Bank located at the Faculty Farm, Faculty of Agriculture and Natural Resources Management, Ebonyi State University.

Determination of the floral characteristics of five Musa genotypes: Female and male flowers of *Musa* genotypes were carefully detached from the inflorescence and taken to the laboratory. The stamen and the pistil were carefully cut out with razor blade. The stigma length was measured by carefully placing the measuring rule on the stigma and reading out the value in centimetres. Stigma width was measured horizontally while the style length was measured by placing the rule straight from the lower to the upper part of the style. Anther length was measured carefully by placing the rule vertically on the anther.

Collection of pollen grains of Musa genotypes: Polleniferous bud were collected before anthesis, buds were excised from the male flowers between 7:30 and 10:30 am and immediately taken to the laboratory. This was to prevent heat from the sun from drying out the sticky pollen grains. Anthers were teased off from the flower buds into five ml of 70 % alcohol in 25 ml beakers. They were squashed with a glass rod. This process breaks the anthers to release the pollen grains. The solutions were sieved with fine network mesh of wire gauze (sterilized under the Bunsen burner to avoid contaminations).The process removes all the plant tissues. The solutions were then centrifuged at 2000 revolution per minute (RPM) for 5 minutes. They were washed twice with distilled water, centrifuged and then decanted again.

Acetolysis: Pollen precipitates were acetolyzed using a modified acetolysis method involving 9:1 conc. sulphuric acid and acetic anhydride for ten minutes (Erdtman,,1971). They were centrifuged, decanted and washed twice with distilled water. The recovered precipitates were stored in vials with ten drops of glycerin for subsequent light microscopy. Temporary slides were prepared, pollen grains were mounted in glycerin jelly and examined using light Olympus CH Trinocular microscope (LM) fitted with 650 IS Cannon Digital Camera at x 400 and x 100 magnifications.

Estimation of potential pollen size, variability and viability of Musa genotypes: Pollen samples were collected from the genotypes between 7:30 and 10:30 am and immediately taken to the laboratory. The pollen grains were manually dislodged from the stamen, spread on a glass slide and stained with acetocarmine with glycerol jelly. The preparation was covered with cover slide and allowed to stand for 24 hours to allow passive uptake of stain. The slide preparations was then observe under bright-field illumination (x 40 magnification) using Leitz Diaplan Binocular Microscope. The diameters of ten randomly selected deeply stained grains were measured with the aid of a graduated eyepiece. The most frequent (n) pollen size, the number of normal or haploid (n), diploid (2n) and giant (4n) pollen grains were recorded per genotype.

Statistical analysis: All data collected were analyzed using the general linear model (GLM) procedure in Statistical Analysis System (SAS), SAS Institute (2000), and Version 9. Means separation was done to determine the differences between genotypes for particular traits.

Results

Floral structure of Musa genotypes: Results indicated significant differences (P < 0.001) among the genotypes for style and anther length. No significant differences were observed for stigma length, stigma width and stigmatic area among the genotypes (Table 1). 'Agbagba' had the longest style (4.31±0.046 cm) which was significantly different from values recorded for PITA 17, FHIA 25 and LBN. The shortest style which was below the mean value for all the genotypes was recorded for PITA 14, an 11TA hybrid (3.82±0.033 cm). Similarly, the longest anther length was recorded for Agbagba (4.81±0.028 cm), followed by PITA 17 (4.33±0.040 cm). The anther length of FHIA 25, PITA 14 and LBN were statistically the same, lower than the mean value and the values recorded for the other two genotypes.

Pollen morphology of Musa genotypes: Result (Table 2) indicated significant differences among the genotypes for big pollen (P < 0.001), moderate pollen (P < 0.01) and apoporium area (P < 0.001). There were no differences among the genotypes for small pollen diameter and length of polycolporate. 'Agbagba' (a landrace genotype) had the biggest pollen (173.2 ± 3.6 µm) which was significantly different from that of PITA 14, 'Lagos banana' and FHIA 25 (Fig. 1). The smallest pollen was recorded for 'Lagos banana' (54.7 ± 3.3 µm), which was significantly different from values obtained for PITA 17, 'Agbagba', and PITA 14 (Table 3).

Table 1: Floral Morphology of the five *Musa* genotypes

Genotype	Stigma Length	Stigma Width	Stigmatic Area	Style Length	Anther Length
Agbagba	0.41±0.010	0.30±0.000	0.12±0.003	4.31±0.046	4.81±0.028
FHIA25	0.41±0.010	0.29±0.018	0.12±0.008	4.14±0.031	4.11±0.031
LBN	0.41±0.010	0.29±0.018	0.12±0.008	4.14±0.031	4.11±0.031
PITA14	0.41±0.010	0.30±0.000	0.12±0.003	3.82±0.033	4.16±0.031
PITA17	0.41±0.010	0.30±0.000	0.12±0.003	4.20±0.000	4.33±0.040
Mean	0.41	0.29	0.12	4.12	4.30

Table 2: Analysis of variance results indicating significant differences among the Musa genotypes for some floral traits studied

Source	DF	Big Pollen Diameter	Moderate Pollen. diameter	Small pollen diameter	Apoporum	Length of colporate
Rep	4	872.0	307.3	92.5***	20.4	3148.8
Genotype	4	8217.1***	1841.8**	1195.1	3472.6***	14.2
Error	240	459.9	431.4	100.0	105.2	51.4
R-Sq		0.2	0.1	0.2	0.4	0.7
CV		13.6	34.4	38.9	35.3	15.2

The highest value for apoporium area was recorded for 'Lagos banana' (37.1± 1.5 µm). This was significantly different (P < 0.001) from values obtained for PITA 14 and PITA 17, while the lowest value was recorded for 'Agbagba' (16.7± 1.0 µm).

Results also indicated that the pollen grains of Lagos banana had apertural type of polycolporate and psilate sculpturing ornamentation with an equatorial shape that was circular (Plate. 2). It had a very thin layer of exine which enclosed the fine elaborate structures on the sexine. The pollen grains of FHIA 25 had polycolporate as its apertural type. The sculpturing type was psilate, and the shape of pollen at equatorial view was circular (Plate 2b). FHIA 25 pollen grains also had two types of apertures as in Lagos banana pollen grains. It had pores that were spheroidal in shape and polycolporate.

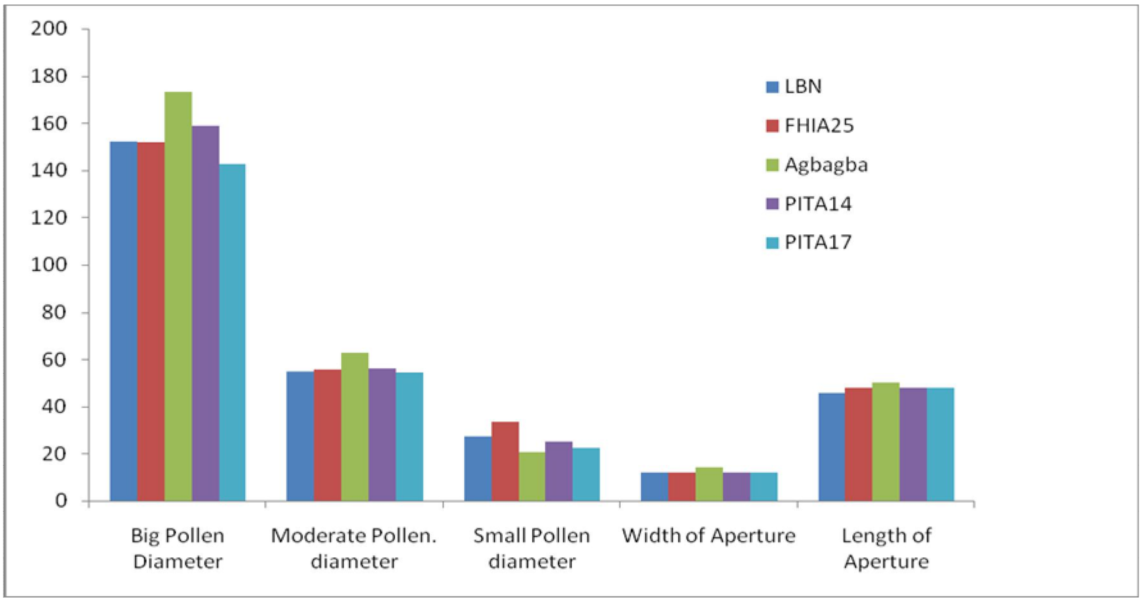


Fig 1: Quantitative data on pollen feature of the five *Musa* genotypes (mean in µm, x 1000).

The pollen grains of PITA 14 had polycolporate apertural type with psilate sculpturing. |Shape of pollen at equatorial view was circular with a very thin exine wall (Plate 2c). The pollen grains of PITA 17 had polycolporate apertural type and psilate sculpturing, while the shape of the pollen at equatorial view was circular. It had very thin exine covered by fine elaborate structures on the sexine. The pollen grains of Agbagba had polyporate apertural type and psilate sculpturing type, while pollen shape at equatorial view was circular with a very thin exine. On the other hand, the pollen grains of Lagos banana, FHIA 25 and PITA 17 were polycolporate, while the pollen grains of PITA 14 and Agbagba were polyporate in nature (Table 4).

Table 3: Mean and standard errors for some floral traits of Musa genotypes studied

	Big Pollen Diameter	Small pollen diameter	Pore diameter	Apoporium	Length of colporate
Agbagba	173.2±3.6	60.7±2.5	20.7±1.2	16.7±1.0	.-
FHIA25	151.8±3.9	59.8±3.0	33.3±1.9	36.2±1.9	47.8±3.1
LBN	152.4±3.1	54.7±3.3	27.1±1.4	37.1±1.8	45.5±2.3
PITA14	168.9±2.4	56.2±3.0	26.0±1.4	26.2±1.1	22.2±
PITA17	142.4±2.0	70.2±2.7	22.4±1.3	28.9±1.1	47.7±2.1
Mean	157.7	60.3	25.9	29.0	40.8

Pollen size variability and viability of Musa genotypes: Results (Table 5) indicated significant differences among the genotypes for number of haploid (n) pollen, diploid (2n) pollen and giant (4n) pollen ($P < 0.001$). The highest number of haploid (n) pollen was recorded for 'Lagos banana' (56.8±2.5), was significantly different from values recorded for PITA 17 and PITA 14. 'Agbagba' had the lowest value of 39.8 ±2.3. On the other hand, the highest number of diploid (2n) pollen was recorded for 'Agbagba' (68.4±1.8), which was not significantly different from values recorded for PITA 17, FIHA 25 and PITA 14.

The highest number of gaint (4n) pollen was recorded for Agbagba (6.3±0.3). This was significantly different ($P < 0.05$) from values recorded for PITA 17 and PITA 14, but not for 'Lagos banana'. The lowest value was recorded for FHIA 25 (3.76±0.29). The highest number of unstained or non viable pollen was recorded for FHIA 25 (55.6± 2.7), followed by 'Lagos banana'. The lowest value for this trait was recorded for PITA 14 (42.4± 2. 1).

Table 4: Qualitative Data on the pollen of the five *Musa* genotypes

Genotypes	Aperture	**N/o Aperture**	**Shape**	**Sculpturing**	**SPPV**
Agbagba	Polyporate	Polypantaporate	Spheroidal	Psilate	Circular
LBN	Polycolporate	Polypantocolporate	Spheroidal	Psilate	Circular
FHIA 25	Polycolporate	Polypantocolporate	Spheroidal	Psilate	Circular
PITA 14	Polycolporate	Polypantocolporate	Spheroidal	Psilate	Circular
PITA 17	Polycolporate	Polypantocolporate	Spheroidal	Psilate	Circular

Table 5: Analysis of variance results indicating significant differences among the Musa genotypes for pollen viability traits studied

Source	Deeply stained	Not stained	Haploid (n) pollen	Diploid (2n) pollen	Giant (4n) pollen
Rep	1203.3**	1365**	523.60	1876***	13.1**
Genotype	527.26	1189.5**	2180.6***	1310***	61.8***
Error	281.14	323.59	276.54	288.56	4.21
R-Sq	0.09	0.12	0.14	0.15	0.24
CV	25.60	37.24	34.99	26.63	44.59

Table 6: Mean and standard errors for pollen viability traits of the five *Musa* genotypes

Genotype	Deeply stained	Not stained	Haploid (n) pollen	Diploid (2n) pollen	Giant (4n) Pollen
Agbagba	65.4±2.5	46.3±2.6	39.8±2.3	68.4±1.8	6.3±0.3
LBN	61.8±2.2	49.8±2.7	56.8±2.5	55.0±3.9	5.3±0.3
FHIA25	66.0±2.4	55.6±2.7	50.4±2.3	65.2±2.0	3.8±0.3
PITA14	70.6±2.3	42.4±2.1	43.6±2.5	64.4±2.2	4.8±1.0
PITA17	63.8±2.7	47.4±2.8	47.6±2.3	65.6±2.1	4.0±0.3
Mean	65.5	48.3	47.6	63.7	4.8

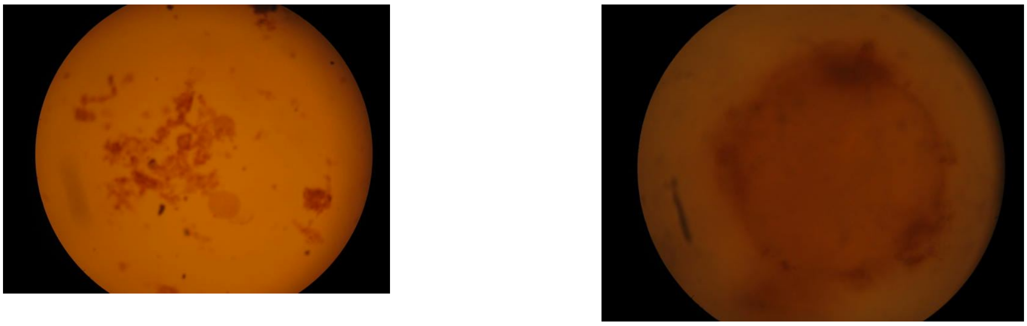
Discussion

The floral parts of *Musa* genotypes comprise the female and male flowers. According to Simmonds (1953, 1959), male and female flowers are morphologically indistinguishable. The flowers have five stamens, a three-lobed stigma and a style. The five stamens in male flowers carries the sticky pollen grains, but is reduced to stameniode in female flowers. The colour of the flowers and the sticky nature of the pollen grains attract both insect and animal pollinators.

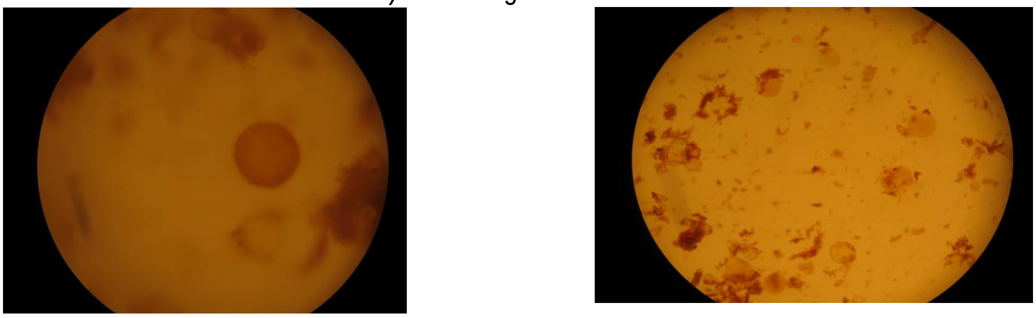
The pollen morphology of the five *Musa* genotypes included in this study was similar except for their apertural types. The close similarity between the pollen morphology of different *Musa* genotypes had been reported by Dafni (2000). Our studies showed that 'Lagos banana', FHIA 25 and PITA 17 had polycolporate pollen grains while PITA 14 and 'Agbagba' had polyporate pollen grains. The presence of polyporate aperture in PITA 14 and 'Agbagba'suggested a higher level of specialization in these species. This observation corroborates an earlier report by Dessein *et al.,* (2005) which noted that the number of apertures were positively correlated with pollen size, since the larger the pollen grain, the larger the surface area where the colpi may be initiated. The report further stressed that more apertures may facilitate higher germination of pollen tube on the stigma and that a possible explanation may be found in the harmomegathy function of the colpi, a process by which the pollen and spores change in shape, to accommodate variations in the volume of the cytoplasm caused by the changes in dehydration. The study concluded that a larger volume requires more apertures to carry out this function.

The pollen grains of the five *Musa* genotypes were circular in shape with a very thin exine. According to Oke *et al.,* (1998), exine is composed of sporopollenin which is chemically stable and resistant to all kinds of environmental damage. Among the five *Musa* genotypes, PITA 14 pollen grains were the most viable pollen grains because they had the highest number of deeply stained pollen. As stated by Fortescue and Turner (2004), diploid *Musa* species produce more viable pollens than tetraploids which in turn produce more viable pollens than triploids. There was also a close range of values for semi viable and dead pollen. Fortescue and Turner (2004), however, reported that viability is only measured in terms of the presence of vital features such as an intact plasma membrane or positive esterase activity. Yaegaki *et al.*, (2002) on the other hand proved that temperature of experiment and time of collection of sample can also lead to death of some pollen and lead to their semi viability.

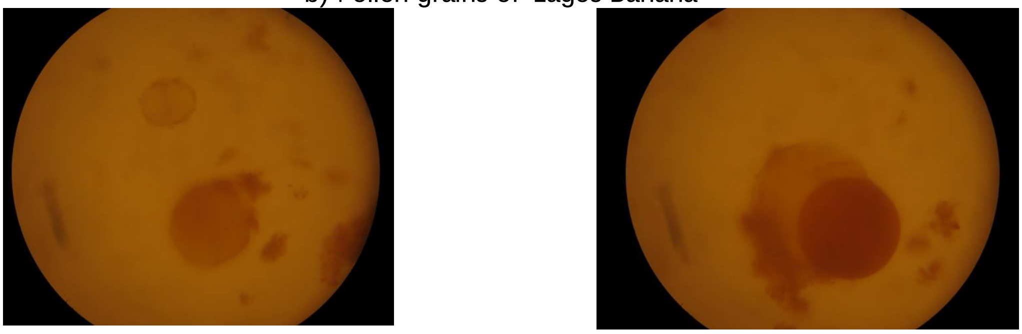
The results obtained in this study are important for improving our understanding in *Musa* reproductive biology. The differences observed from the length, breath and nature of the style and anther was significant among the various genotypes. The level of stainability of the pollen grains of the genotypes is also indicative of their viability. For this trait, the Agbagba variant used in this study will be further investigated as possible candidate for breeding programs aimed at improving landrace plantain genotypes since it showed higher level of stainability and thus viability.



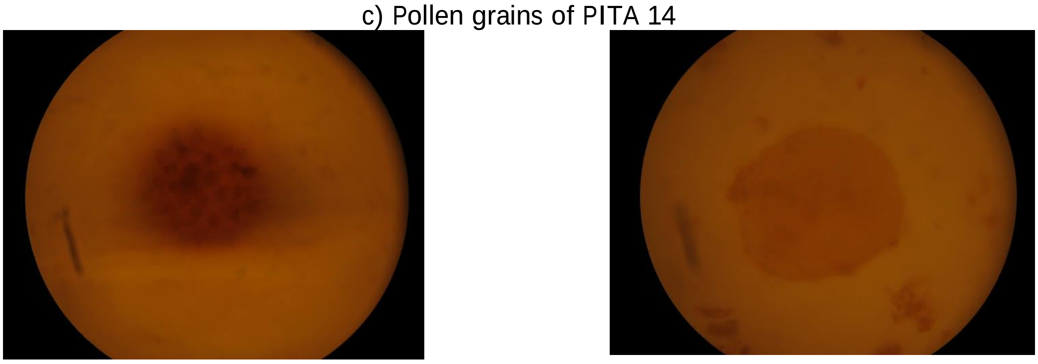
a) Pollen grain of FHIA 25



b) Pollen grains of ‘Lagos Banana’



c) Pollen grains of PITA 14



d) Pollen grains of PITA 17

Figure 2: Sizes of the pollen grains of five Musa genotypes studied

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