International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor (2018): 7.426

Incidence of Irregular Sporads in Some *Musa* Accessions

Adeleke, Martina T. V.

Department of Plant Science and Biotechnology, Rivers State University, Nkpolu, Port Harcourt, Nigeria

Abstract: ***Musa species have complex cytogenetic structure, resulting in extensive chromosomal rearrangements during meiosis. This is mainly because Bananas are derived from interspecific and intersub specific hybridization. Most banana cultivars are triploid (2n=3x=33) interspecific hybrids between M. acuminata (A genome) and M. balbisiana (B genome). They are characterized by low male and female fertility. The uneven chromosome number gives rise to meiocytes with unequal chromosome number. The study shows univalents at metaphase I, precocious movement of chromosomes, bridges and lagging chromosomes at anaphase I. Incidence of univalents gave rise to poly- and irregular spore formation.***

Keywords: Musa species, sporads, univalent, laggards, chromosomes

1. Introduction

Bananas and plantains (*Musa* spp. L) are one of the major staple food and cash crops today, especially in the tropics and sub-tropics [11]. They provide a valuable model for studying chromosomal rearrangements [12].Most banana cultivars are derived from *Musa acuminata* (2n = 2× = 22, A genome), sometimes combined with *Musa balbisiana* (2n = 2× = 22, B genome). *M. acuminata* is divided into six to nine subspecies viz. *banksia, burmannica, malaccensis, microcarpa, zebrina, burmannicoïdes, truncata*, *siamea*, and *errans*, which diverged following geographical isolation in distinct Southeast Asian continental regions and islands [5; 15]. The currently accepted domestication scenario suggests that human migrations, probably during the Holocene, led to contacts between these subspecies through the transport of plant material [16]. This resulted in the emergence of intersubspecific hybrids with reduced fertility [9; 10; 19]. Early farmers would then have selected parthenocarpic diploid and triploid hybrids producing fruit with high flesh and low seed content.

Nearly sterile edible diploids produced triploid progeny (AAA; 2n = 33): the product of female restitution with 22 chromosomes (AA) in the female gamete and 11 chromosomes (A) from the haploid pollen[20]. The AAA triploids have larger fruits and are more vigorous, productive and hardier than the diploids; they are normally parthenocarpic and male sterile. Being more attractive to the grower, the AAA triploids were multiplied at the expense of the diploids. With time, natural hybridization occurred between *M. acuminata* (AA and AAA) and wild-seeded diploid *M. balbisiana* (BB; 2n = 22) to produce hybrid progeny with the genomes AB, AAB and ABB. The triploid hybrids could have been formed from diploid AB hybrids which yield AAB and ABB types on backcrossing, or by female restitution in AA clones with *balbisiana* pollen to give AAB types, followed by backcrossing to give ABB types. There are also natural ABBB tetraploid clones [18]. Banana and plantain production depends upon a limited number of landraces selected by man from the natural germplasm [14]. The inability to replace these landraces by cultivars obtained through cross-breeding creates the increased risk of epidemic disease such as Black Sigatoka among genetically similar cultivars. Consequently, improvement programs have concentrated almost exclusively on resistance breeding. Difficulty in genetic improvement has been attributed to a lack of useful genetic variability, parthenocarpy and low levels of female fertility. The commercial *Musa* cultivars are mainly triploids (2n=3x=33). *Musa* species exhibit low seed set and poor seed germination, this is because of the low male and female fertility [21], and some are completely sterile. Pairing irregularities due to uneven chromosome number of 33, as well as hybridity between subspecies and mutations, alter the frequency and distribution of meiotic exchanges necessary for successful meiosis;thus making chromosome segregation errors imminent at meiosis I [3].

Erratic movement of chromosomes during anaphase [22], and the presence of univalents, amongst other causes, all contribute to infertility in this genus. Hence plant multiplication is done mainly by vegetative means. Banana improvement programs in breeding for resistance, use wild diploid species to introgress resistance genes into landraces; firstly by crossing a 3x landrace with a 2x wild diploid to produce tetraploids (4x). Selected tetraploids with desired traits are then crossed with wild or improved diploids to produce secondary triploids [17]. These crosses produce unexpected ploidy frequencies and undesired traits due to erratic chromosome segregation at meiosis. This paper reports the various abnormalities observed during sporad formation of some *Musa* accessions from the *Musa* field gene bank of International Institute of Tropical Agriculture (IITA) high rainfall station at Onne, South-eastern Nigeria.

2. Materials and Methods

Plant material

The plants used in this study included 35 accessions consisting of meiotic mutants selected from diploid, triploid and tetraploid populations of the wild species and landraces of *Musa*. The plants were obtained from the germplasm bank at the high rainfall station at Onne, South-eastern Nigeria. Only plants with available male buds were used for meiotic and sporad analysis.

Chromosome preparation

Meiotic chromosome spreads were prepared according to the procedure described in Adeleke et al. [1]. Chromosomes were photographed in a Leitz Diaplan microscope using Ilford PANF 50 film.

Sporad Counts

Freshly harvested male buds harbouring tetrad suspensions were selected according to the method of Adeleke et al. [1]. The suspension was squeezed from the anther unto a clean slide. A drop of water was added to the suspension to keep the sporads in solution while counts of monads, diads, triads tetrads, pentads and more were made under the light microscope using a 6.3/0.2 objective and 12.5x eye piece. The sporads were also photographed using a Leitz Diaplan microscope using Ilford PANF 50 film.

3. Result and discussion

The meiotic chromosome spreads showed quite a number of the accessions with univalents at the metaphase, as well as bivalents, trivalents and even some quadrivalents (Fig. 1-5). Chromosome pairing studies provided early indications as to the genome relationships between polyploid species and diploid relatives [13]. Furthermore, Dewey [7] very explicitly states that like (homologous) chromosomes pair and unlike (non-homologous) chromosomes do not. Therefore, homologous chromosomes are similar and pairing behavior is required to infer homology. It is however now known that pairing can be controlled by genes. In the *Musa* accessions studied, no single accession gave just one pairing pattern at metaphase I (Table 1).

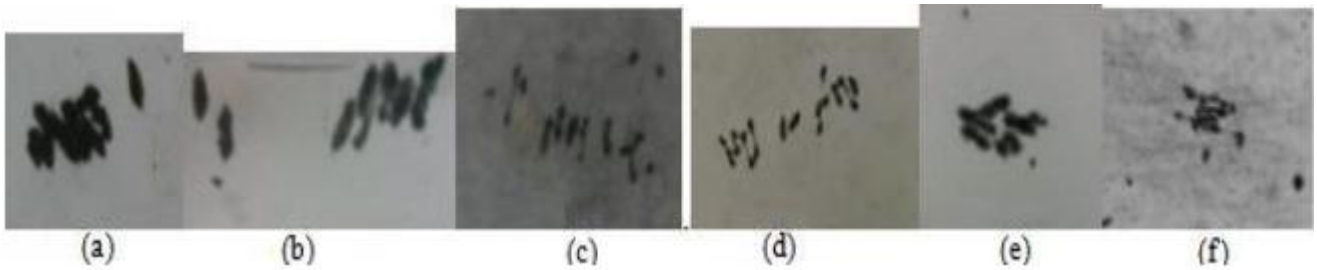

Figure 1: Meiotic metaphase I chromosome of DiploidBananas; a-d: AA; e: BB; f: AB
(a) Calcutta 4 (b) Pitu (c) Pisang jari buaya (d) 4600-12 (e) *M. balbisiana (*f) Kamaramasengi

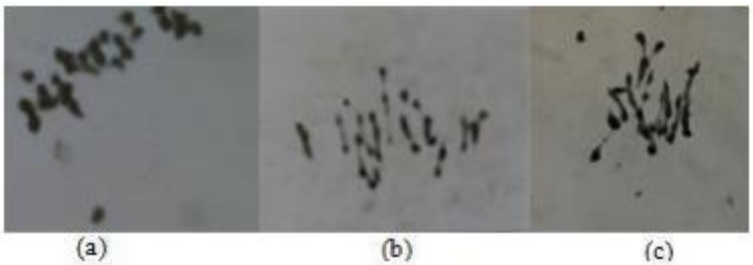

Figure 2: Metaphase I in Triploid Bananas AAA (a) High gate (b) Igisahira gizanzwe (c) Igumba

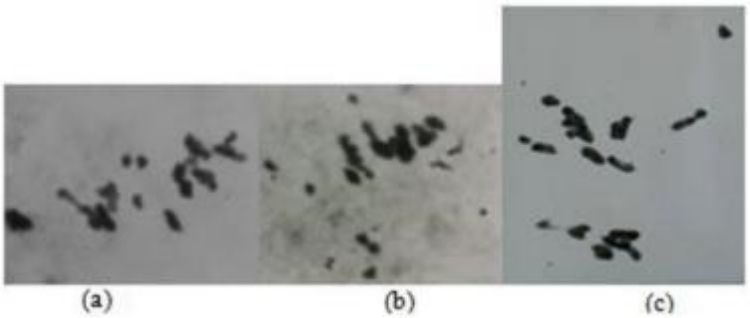

Figure 3: Metaphase I in Plantains AAB (a) (Obino L'ewai) (b) French Reversion (c) O. Ntanga

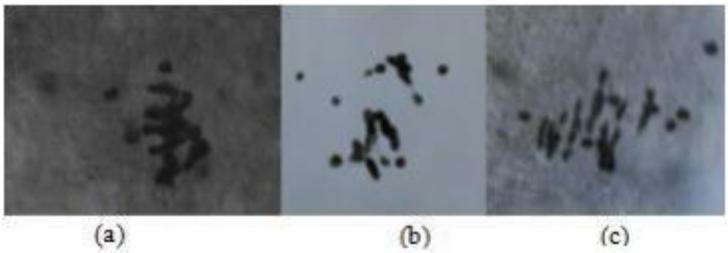

Figure 4: Metaphase I in Cooking Bananas (ABB) (a) Cardaba (b) Maduranga (c) Fougamou

Figure 5: Metaphase I in Tetraploids (a) 1378 (Fougamou X Balbisiana) ABBB (b) 612-74 (Bluggoe X C4) AAAB

Table 1: Chromosome association at metaphase I in pollen mother cells of the different *Musa* accessions and their values per pollen mother cell

Musa Accessions	2n no.	Genome	I	II	III	IV	V
Calcutta 4 (C4)	22	AA	0.22	8.42	1.22	0.32	-
P. lilin	22	AA	0.16	9.42	0.60	0.30	-
Pitu	22	AA	0.14	8.34	0.82	0.68	-
Borneo	22	AA	0.50	9.74	0.46	0.16	-
M. balbisiana10852	22	BB	0.20	10.24	0.28	0.12	-
M. balbisiana HND	22	BB	0.18	9.30	0.62	0.12	-
Eti Kehel	22	BB	0.26	9.56	0.82	0.04	-
Singapuri	22	BB	0.34	9.38	0.86	0.08	-
Kamaramasengi	22	AB	1.20	7.50	0.60	1.00	-
Ney Poovan	22	AB	1.60	7.86	1.32	0.18	-
Cardaba	33	ABB	3.74	9.14	3.66	-	-
Saba	33	ABB	1.83	8.17	4.50	0.33	-
Fougamou	33	ABB	1.84	10.24	3.78	-	-
Ice cream	33	ABB	1.02	3.06	8.60	0.02	-
Obino L'ewai	33	AAB	1.18	10.54	3.34	0.18	-
French Reversion	33	AAB	1.46	13.56	1.18	0.22	-
Bobby Tannap	33	AAB	1.70	12.10	2.18	0.14	-
Igisahira gizanzwe	33	AAA	1.22	5.10	6.02	0.82	0.18
High gate	33	AAA	1.64	8.66	3.46	1.14	0.02
Yangambi Km5	33	AAA	1.10	11.58	2.62	0.28	-
2829-62 (Bob. T X C4	22	AA	0.24	9.00	1.56	0.22	-
4400-8 ,, ,,	22	AA	0.18	9.68	0.74	0.06	-
2625-5 ,, ,,	22	AA	0.16	4.94	0.44	0.24	-
9007-4 ,, ,,	22	AA	0.38	8.92	1.10	0.12	-
1448-1 (Obino L'ewai)	22	AA	0.17	9.60	0.75	0.10	-
1549-5 ,, ,,	22	AB	0.19	9.88	0.69	-	-
612-74 (Bluggoe X C4)	44	AAAB	1.00	11.48	5.28	0.86	0.08
1378 (Fougamou X Balb)	44	ABBB	1.14	13.82	2.90	1.28	0.12

The presence of large chromosome structural variations within *M. acuminata* was proposed by cytogeneticists on the basis of the observation of chromosome pairing irregularities at meiosis in hybrids between *M. acuminata* accessions [8; 9; 6; 10; 19]. Cytogenetic studies have shown that chromosomal pairing at meiosis in *Musa acuminata* is generally regular in bivalents within subspecies, but irregular with some multivalents and univalents in hybrids between subspecies [8; 9; 6; 10; 19]. Chromosomal structural variations between subspecies have been put forward to explain those irregularities.

Many crops such as banana are derived from interspecific hybridization; and many banana cultivars are triplod (sometimes diploid) interspecific hybrids between M. acuminata (A genome) and M. balbisiana (B genome)[4]. Meiotic studies by Agarwal [2] in diploid *Musa* species suggest that the level of homology between the A and B genomes is highly variable. The triploids had higher levels of univalents and trivalents per pollen mother cell (PMC) than the diploids (Fig. 2, 3, 4, Table 1). Here two tetraploids also have high trivalent values per PMC (Fig. 6, Table 1). The tetraploid hybrid 612-74 has a value as high as 5.28, depicting its AAAB genome; and it still has a relatively high value of 11.48 for bivalents per PMC. This suggests two sources of the A genome in this hybrid. In this study, chromosomal associations were mainly of univalents to quadrivalents and very few pentavalents (Igisahira gizanzwe, High gate, 612-74 and 1378) were seen in all the representative Musa accessions studied.

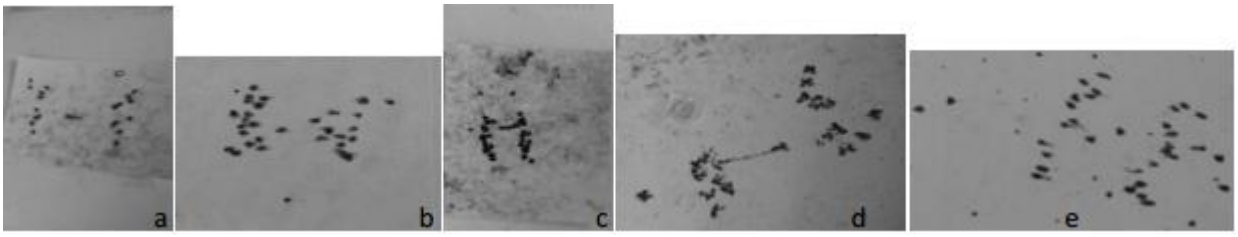

Figure 6: Anaphase I in some *Musa* accessions showing bridges and laggards.
a) 4400-8 (AA); (b) French Reversion (AAB); (c) Lepchang kut (ABB); (d) C4 (AA); (e) Igisahira (AAA)

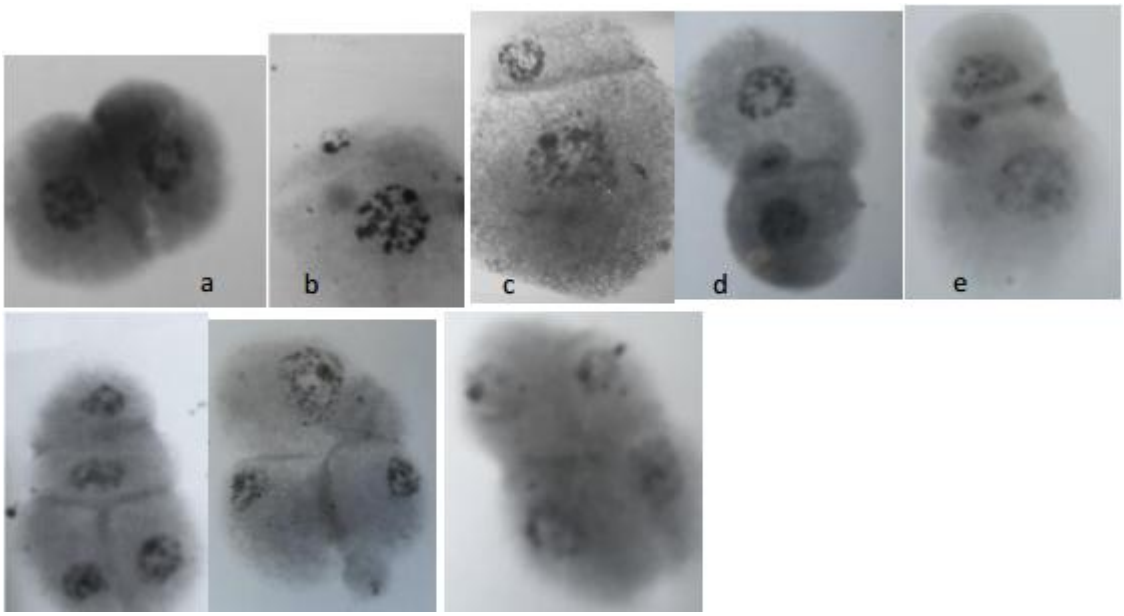

Figure 7: Dividing pollen mother cells of some *Musa* accessions with different configurations of potential sporads: b and c show unequal division of nuclei; d and e show laggard at metaphase plate surrounded with cytoplasm to form potential spores; f, g and h are different configurations of tetrad spores.

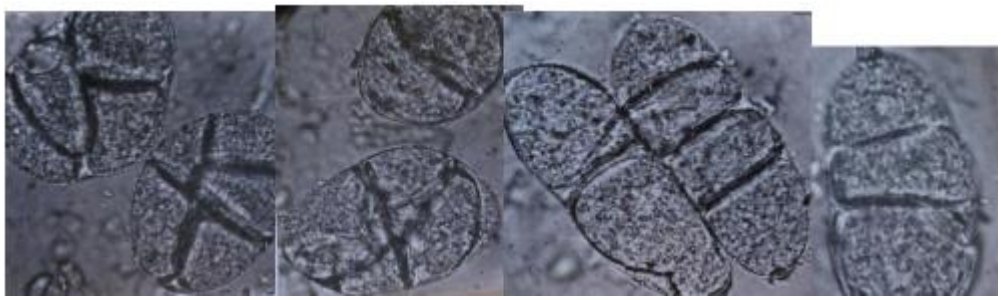

Figure 8: Polyspory in *Musa*

The occurrence of univalents in all the Musa accessions studied serves as a pointer to the erratic chromosome movements observed generally in this genus at anaphase [22]. Univalents often do not get on the metaphase plate but may be arranged randomly along the spindle. These laggards that fail to get on the metaphase plate may move to the nearer pole ahead or behind the chromosomes that behave normally at anaphase (Fig. 6). In addition, cytogenetic aberrations at anaphase I such as lagging chromosomes and anaphase bridges were also observed (Fig. 6). Where there is a chiasma in a paracentric inversion, a chromatid bridge is formed between the anaphase groups of chromosomes (Fig. 6). A fragment is usually left at the metaphase plate at anaphase (Fig. 6a). The laggards and univalents were excluded from the nuclear material of daughter cells during spore formation, and were enclosed by cytoplasm becoming potential spores (Fig 7d and e). This study showed that irregularities in chromosome pairing (which is an indication of genome relationship) at metaphase I in hybrids can be traced to laggards and anaphase bridges; and this also can be traced to the rounding up of these chromosomes with less than the haploid number of chromosomes, as spores.

4. Acknowledgements

This research was supported with funds from the Directorate General for International Cooperation (DGIC, ex BADC), Belgium.

References

[1] Adeleke, M. T. V., Pillay, M. and Okoli, B. E. 2002. A novel method for examining meiotic chromosomes in Musa L. HortScience 37: 959–961.
[2] Agarwal, P. K. 1988. Cytogenetical investigations in Musaceae. III. Meiotic studies in diploid Musa species and banana varieties of India. Cytologia, 53: 359-363.
[3] Baker, B. S., Carpenter, A. T. C., Esposito, M. S., Esposito, R. E. and Sandler, L. 1976. The genetic control of meiosis. Annu. Rev. Genet. 10: 53–134.
[4] Baurens, FC., Martin, G., Hervouet, C., Salmon, F., Yohomé, D., Ricci, S., Rouard, M., Habas, R., Lemainque, A., Yahiaoui, N., and D'Hont, A. 2019. Recombination and Large Structural
[5] Variations Shape Interspecific Edible Bananas Genomes, Molecular Biology and Evolution, 36 (1): 97–111,
[6] Daniells, J. 2001. Musalogue : A catalogue of Musa germplasm: diversity in the genus Musa. Biover Int . 213.
[7] Dessauw, D. 1987. Etude des facteurs de la stérilité du bananier (Musa spp.) et des relations cytotaxinomiques entre M. acuminata Colla et M. balbisiana Colla. Fruits, 43: 539-588/ 615- 638/ 685-700.
[8] Dewey, D. R. 1982. Genomic and phylogenetic relationship among North American perennial Triticeae. Pp. 51-81 in Estes J. R. et al. (eds), Grasses and grasslands. University of Oklahoma Press, Norman.
[9] Dodds, K. S. 1943. Genetical and cytological studies of Musa. V. Certain edible diploids. J Genet. 45:113–138.
[10] Dodds, K. S., Simmonds, N. W. 1948. Sterility and parthenocarpy in diploid hybrids of Musa. Heredity 2:101–117.
[11] Fauré, S. , Bakry, F. and González de Leon, D. 1993. Cytogenetic studies of diploid bananas. In: Breeding banana and plantain for resistance to diseases and pests . Montpellier: CIRAD-FLHOR. p. 77–92.
[12] Lescot, T. 2014. La diversité génétique des bananiers. FruiTrop 221:98.
[13] Martin, G., Carreel, F., Coriton, O., Hervouet, C., Cardi, C., Derouault, P., Roques, D., Salmon, F., Rouard, M., Sardos, J., Labadie, K., Baurens, F. and D'Hont, A. 2017. Evolution of the banana genome (*Musa acuminata)* is impacted by large chromosomal translocations. Mol Biol Evol . 34 (9): 2140–2152.
[14] Moore, G. 2000. Cereal chromosome structure, evolution and pairing. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51: 195-222.
[15] Ortiz, R., Ferris, R. S. B., and Vuylsteke, D. 1995. Banana and plantain breeding, pp110-146. In: Gowen, S. (ed).
[16] Banana and plantain. Chapman& Hall, U.K.Perrier, X., Bakry, F., Carreel, F., Jenny, C., Horry, J-P., Lebot, V., Hippolyte, I. 2009. Combining biological approaches to shed light on the evolution of edible bananas. Ethnobot Res Appl. 7:199–216.
[17] Perrier, X., De Langhe, E., Donohue, M., Lentfer, C., Vrydaghs, L., Bakry, F., Carreel, F., Hippolyte, I., Horry, J-P., Jenny, C., Lebot, V., Risterucci, A-M., Tomekpe, K., Doutrelepont, H., Ball, T., Manwaring, J., Maret, P. and Denham, T. 2011. Multidisciplinary perspectives on banana (Musa spp.)domestication. Proc Natl Acad Sci U S A. 108 (28):11311–11318.
[18] Pillay, M., Tenkouano, A. and Hartman, J. 2002. Future challenges in Musa breeding. In: Kang, M. S. (ed.). Crop improvement for the 21st century. Food products press, New York. pp
[19] Purseglove, J. W. 1988. Tropical Crops: Monocotyledons. Longman scientific & technical, England. Pp 345-370.
[20] Shepherd, K. 1999. Cytogenetics of the genus Musa. International Network for the Improvement of Banana and Plantain, Montpellier, France.
[21] Simmonds, N .W., Shepherd, K. 1955. The taxonomy and origins of the cultivated bananas. Bot J Linn Soc55 (359):302–312.
[22] Vuylsteke, D. R., Swennen R. L., Ortiz R. 1993. Development and performance of black sigatoka-resistant tetraploid hybrids of plantain Musa spp., AAB group). Euphytica 65 (1):33–42.
[23] Wilson, G. B. 1946. Cytological studies in the Musae.II. Meiosis in some diploid clones. Genetics, 31: 475-482