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A Preliminary Cytological Study of 2n Pollen Formation in Musa

M. T. V. Adeleke[1*], M. Pillay[2], B. E. Okoli[3]

[1]Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Nigeria.

[2]Department of Biotechnology, Vaal University of Technology, Vanderbijlpark, South Africa.

[3]Department of Plant Science and Biotechnology, University of Port Harcourt, Port Harcourt, Nigeria.

Current Musa breeding strategies are complex and time consuming involving the selection of tetraploids from 3x-2x crosses. The tetraploids are crossed with diploids to produce secondary triploids. Considering the very low hybrid seed set, routine embryo rescue procedures of hybrid seeds and the long growth cycle of banana, it takes approximately 10-12 years to produce an acceptable banana hybrid. The banana breeding process could benefit tremendously if triploid bananas could be obtained directly from 2x-2x crosses through the process of unilateral polyploidization. There are few reports on the mechanisms involved in 2n pollen production in Musa. This study investigated the type of meiotic irregularities that lead to 2n pollen formation in diploid, triploid and tetraploid Musa accessions using cytological analyses. The results showed that aberrations in cytokinesis and karyokinesis during microsporogenesis are possible mechanisms for 2n pollen formation in Musa. The meiotic aberrations described in this study have implications for Musa breeding. It appears that 2n pollen formation in Musa occurs via both FDR (first division restitution) and SDR (second division restitution). FDR is said to be more promising in transferring more heterozygosity from parents to offspring.

Key words: *Musa spp., abberrations, chromosomes, cytokinesis, karyokinesis.*

INTRODUCTION

Although meiosis is considered as a highly conservative process that leads to a reduction in the chromosome number in the gametes, mutations in the genes controlling the process lead to abnormalities some of which can produce 2n gametes in plants (Pagliarini 2000) [1]. The formation of 2n or diplogametes is a common feature in many plant species including wild potato (Camadro et al., 2008), *Hibiscus* (van Laere et al., 2009), *Begonia* (Dewitte et al 2010), *Turnera sidoides* (Kovalsky and Neffa 2016), *Avena ventricosa* (Nicoloudakis et al., 2018), lemon (Xie et al., 2019) and Cymbidium (Zeng et al., 2020). The production of 2n gametes in plants is considered to be a dominant process in the origin of polyploid crop species (Harlan and de Wet, 1975) as well as the development of cultivars (Lim et al., 2001). More than 70% of flowering plants are polyploids (Leitch and Bennett, 1997) [2-6]. Musa is a polyploid complex that comprises diploid species and triploid and tetraploid accessions that originated from

*Corresponding author: E-mail: mtadeleke@yahoo.co.uk

inter- and intraspecific hybrids between *M. acuminata* and *M. balbisiana* . The most common way of detecting 2n pollen is searching for large pollen size since large pollen has frequently been attributed to 2n pollen in many genera (Van Laere et al., 2012) [7]. Gametes with unreduced chromosome numbers have been reported to occur naturally at a low frequency in Musa (Dodds, 1943; Dodds and Simmonds, 1946; Sathiamoorthy and Balamohan, 1993). The normal pollen size for two wild banana species *M. acuminata* and *M. balbisiana* was reported to be 104 + 1 µ and 94 + 2 µ, respectively (Ortiz, 1997) [8].

Our previous study showed that pollen sizes ranged from 84.30 to 107.20 µ in 24 diploid and triploid banana accessions (Adeleke et al., 2004). Dodds (1943) indicated that pollen with a diameter of 129 µ or was considered haploid (n) while the average diameter of 2n pollen was 147.6 µ. Ploidy analysis of hybrids derived from 2x-2x crosses in banana showed that some plants are triploids implying the formation of 2n gametes in Musa. Unreduced gametes are important in plant breeding as an efficient method to transfer germplasm from lower to higher ploidy levels (Vorsa and Bingham, 1979; Van Laere et al., 2012) [9-11].

Diseases and pests such as black Sigatoka, fusarium wilt, nematodes and weevils affect banana production throughout the world. Breeding is regarded as the most economical means of producing disease and pest resistant bananas. Current Musa breeding strategies are complex and time consuming involving the selection of tetraploids from 3x-2x crosses. The tetraploids are crossed with diploids to produce secondary triploids (Pillay et al., 2002). Considering the very low hybrid seed set, routine embryo rescue procedures of hybrid seeds and the long growth cycle of banana, it takes approximately 10 -12 years to produce an acceptable banana hybrid. The banana breeding process could benefit tremendously if triploid bananas could be obtained directly from 2x - 2x crosses through the process of unilateral polyploidization [12-16].

The formation of 2n gametes occurs via the phenomenon of nuclear meiotic restitution. Nuclear meiotic restitution is defined as the formation of a single nucleus with unreduced chromosome number in place of two nuclei with reduced chromosomes numbers, owing to the failure of either the first or second meiotic division (Ramanna, 1979) [17]. A number of meiotic abnormalities related to spindle formation, spindle function and cytokinesis are considered to responsible for the formation of 2n gametes in several crop plants (Van Laere et al 2012; De Storme and Geelen 2013) [18,19]. They include parallel and tripolar spindles, premature cytokinesis I and II (pc I and pc II) that lead to either first division or second division restitution (FDR and SDR, respectively) (Van Laere et al., 2012). There is now evidence for genetic control of 2n gamete formation in plants (De Storme and Geelen 2013). Investigations into the mechanisms of 2n [20].

Gametes in Musa are limited. Second division restitution was postulated to be involved in megasporogenesis of plantains by Dodds and Simmonds (1946), Hutchinson (1966), Ortiz and Vuylsteke (1994) and Ortiz et al., (1995). Technical difficulties of staining Musa chromosomes have hindered studies concerning the mechanisms of 2n gamete formation. However, new techniques using silver staining have made it possible to investigate 2n pollen formation in Musa (Adeleke et al., 2002) [21-15].

The objective of this research was to investigate the type of meiotic irregularities leading to 2n pollen formation in diploid, triploid and tetraploid Musa accessions using cytological analyses. Our results showed that aberrations in cytokinesis and karyokinesis during meiosis are possible mechanisms for 2n pollen formation in Musa.

MATERIALS AND METHODS

Plant materials

The plants used in this study included 12 accessions of *M. acuminata* Colla. (representatives of the AA genome combination), 6 AAA, 3AAB, 3 ABB triploid landraces, and 7 plantain-banana diploid hybrids, and 2 tetraploid cooking banana-banana hybrids. The female plantain parents of the hybrids were AAB landraces – ‘Bobby Tannap’ and ‘Obino L’ewai’ (French plantains), and ABB landraces-Bluggoe’ and ‘Fougamou’. The male parent was mainly the wild diploid fertile seeded banana *M. acuminata spp. burmannicoides* Calcutta 4 (De Langhe and Devreux, 1960), with the exception of *M. balbisiana* that was crossed with ‘Fougamou’. Only four accessions ‘P.lilin’, ‘High gate’, the diploid hybrid 4600-12, and ‘Pisang Jari Buaya’ showed meiotic abnormalities during pollen formation. Normal meiosis was observed in the other accessions used in this study. Therefore only data for those plants that showed abnormalities are discussed. In this study giant pollen grains were regarded as 2n pollen [26].

Slide preparation

Chromosome spreads from microsporocytes were prepared according to the procedure described in Adeleke et al. (2002) [27]. Briefly, anthers from young male buds were fixed in 3:1 ethanol-acetic acid solution with 1% ferric chloride as a mordant for 18-24 hours at 4°C. The contents of the anther lobes were squeezed out with the aid of a dissecting needle into a drop of LB01 buffer (15 mM Tris, 2mM Na EDTA, 80mM KCl, 20mM NaCl, 0.5mM spermine, 15uM mercaptoethanol, 0.1% Triton X-100, pH 7.5 (Dolezel et al., 1989). The cells were pipetted into a microcentrifuge tube, rinsed several times in citrate buffer, pelleted and digested in an enzyme mixture (5% cellulase, 1% pectinase and

1% pectolyase prepared in citrate buffer, pH 4.5), and

solution was placed on a clean slide and a drop or two of freshly prepared 3:1 ethanol-acetic acid placed over the cells shortly before the smear dried completely. The quality of the slide was assessed by observation in a phase contrast microscope. The best slides were air-dried and stained with silver nitrate according to the procedure described in Lacadena et al., (1984), except that incubation was done for 2-4 min. The slides were made permanent by treating in xylene for 30 min and then mounting in DPX [28].

Photography

Chromosomes were photographed in a Leitz Diaplan microscope using Ilford PAN F 50 film.

RESULTS AND DISCUSSION

One of the insights obtained from this study is that 2n pollen formation in Musa may be due to aberrations in cytokinesis and karyokinesis during microsporogenesis [29]. The meiotic process in dividing pollen mother cells is usually very regular whereby the nuclear content of the cells first divide and move to opposite poles within the cell. This stage is followed by an equational division of the cytoplasm followed by the formation of a cell wall that separates the divided nuclear material. This procedure takes place in both the first and second meiotic divisions, except that the nuclear material that separates in meiosis I are homologous chromosomes, while those in meiosis II are sister chromatids [30]. The whole process, therefore, should result in four daughter cells (pollen) with equal nuclear contents. In this study, we identified three types of aberrations during microsporogenesis in Musa. One type involved unequal cytoplasmic division after meiosis II. Figure 1 shows that the dyad comprises a very large cell and a small cell with corresponding large and small nuclei, respectively. This type of aberration was observed in 'Pisang Lilin' (AA) a wild diploid banana and 'High Gate' (AAA), a dwarf mutant of 'Gros Another type of aberration illustrated in Figure 2 which show s all the nuclear material moved to only one of the two daughter

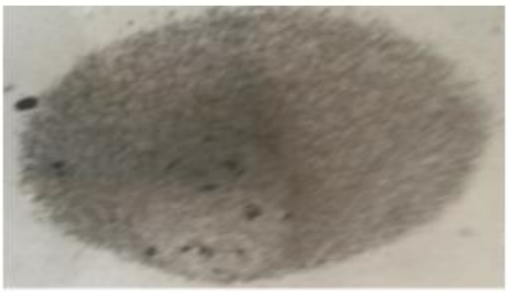

Figure 2: Aberration in meiosis showing one nucleated and one enucleate cell.

were washed again in citrate buffer and re-suspended cells leaving the other cell enucleate. There was equal division of the cytoplasm in meiosis I and one of the daughter cells appears to be undergoing a second nuclear division. There is no evidence of cytokinesis.

Michel', both of which have been classified as 2n pollen producers (Ortiz, 1997). Judging from size alone, the larger cell most likely represents a 2n gamete while the smaller cell appears to be a normal reduced gamete. The exact mechanism leading to the formation of the large and small cells was not evident in this study. Parallel spindle formation has been noted as one mechanism for 2n pollen grain formation (Mok and Peloquin, 1975). On the contrary, Carputo et al., (1995) reported, "parallel spindle is a necessary, but not sufficient condition for the formation of dyads". They also suggested that the presence of other mechanisms acting at the cytokinesis level could influence the formation of dyads. Failure of cytokinesis has been reported to lead to 2n gametes in *Paspalum* (Pagliarini et al., 1999), orchid (Storey, 1956) and Agave spp (Gomez-Rodriguez et al., 2012), while Ramanna (1974) also reported that aberrant cytokinesis can lead to dyad formation. This may also be true for Musa although we were not able to observe any parallel spindles in this study [31-34] (Figure 1).

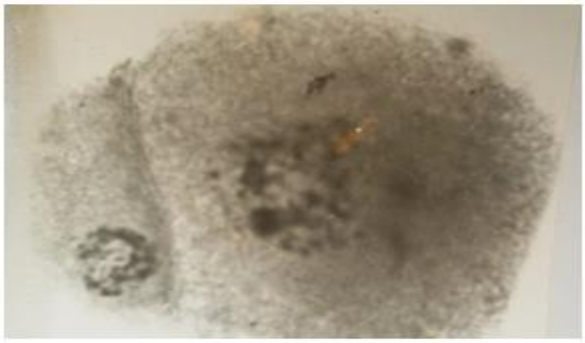

Figure 1: Abnormal cytokinesis in telophase II of meiosis showing large and small cell.

The third type of meiotic abnormality was observed in cells of the wild diploid 'Pisang jari buaya' and is illustrated in Fig 3. In this case the pollen mother cell had divided into two daughter cells that showed lack of synchrony in karyokinesis in one cell and absence of cytokinesis in the other. The nucleus in one of the two cells was divided into two but there was no evidence of cytokinesis. The cell had two nucleoli implying that the cell had a 2n number of chromosomes. In general, an interphase or telophase nucleus has a single nucleolus [35].

The second cell showed no indications of undergoing karyokinesis.

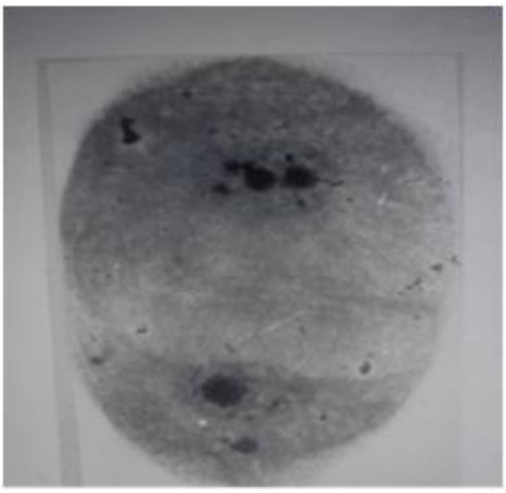

Figure 3: Photomicrograph showing asynchronous karyokinesis and cytokinesis.

The meiotic aberrations described in this study have implications for Musa breeding. Banana breeding is difficult because of triploidy in cultivated accessions, low male and female fertility and differences in ploidy level that hampers the introgression of desirable characteristics from wild species into cultivated bananas (Pillay et al., 2002) [36-38]. Bastiaanssen et al. (1998) suggested that it is better to breed a ploypoid crop species, such as the potato, at the diploid level in which the patterns of inheritance would be straighforward, the selection process more efficient and introgression of characters will be possible. Tezenas du Montcel et al. (1996) and Rowe and Rosales (1996) also advocated (Figure 3).

There is a high degree of expected co-ordination between karyokinesis and cytokinesis in the normal course of meiosis. Meiosis involves specific cytological features and integrated events controlled by a large number of generally dominant genes which are stage-, site- and time specific (Taschetto and Pagliarini, 2003). However, this orderly process can be disrupted by meiotic mutations and is also affected by the environment (Veilleux, 1985). In this study, we reported aberrations observed in both cyto and karyokinesis in Musa. These aberrations offer an explanation for the formation of 2n pollen production in Musa. However, one has to be aware that the identification of the mechanisms leading to unreduced gametes is a complex issue because different individuals in the same species that the initial improvement of bananas should be carried out at the diploid level. Although diploid bananas present many interesting breeding characteristics, they are not easily accepted by consumers because are generally smaller than triploids and are non- parthenocarpic. The formation of 2n gametes in Musa opens up new opportunities for banana breeders to breed at the diploid level and then restore the triploid condition by making use of 2n gametes via unilateral (2x-x) polyploidization.

Whether the 2n gametes in Musa are formed via FDR (first division restitution, failure of 1st division) or SDR (second division restitution, failure of 2nd division) also present important opportunities for banana breeding. Genetic theory has demonstrated that FDR is more efficient than SDR for transferring heterozygosity from diploid parents to the tetraploid progeny (Bingham, 1980). Mendiburu and Peloquin (1977) reported that 2n gametes formed by FDR theoretically transmitted approximately 80% of parental heterozygosity to polyploid offspring, whereas SDR transmitted approximately 40%. First division restitution gametes retain the parental genotypes to a large extent and are largely homogeneous while second division restitution gametes do not retain the parental genotypes and are largely heterogeneous (Ramanna, 1979). The meiotic aberrations in Musa illustrated in this study represent both FDR (Fig 2) and SDR (Fig 1 and Fig 3). The dyad cell that retains all the nuclear material (Fig. 2) would transmit more heterozygosity because homologous chromosomes remain together in the same daughter cell. If the two nucleoli in Fig. 3 remain in one cell, that cell would have the 2n number of chromosomes. This would represent SDR because it contains 2 copies of sister chromatids of each chromosome and it will therefore not transmit much heterozygosity as in FDR.

CONCLUSION

Produce 2n gametes through different cytological mechanisms, and more than one mechanism may operate within an individual plant (Parrot and Smith, 1984, Werner and Peloquin, 1991; Souza et al., 1999). In addition 2n gamete formation in Musa is affected by high solar radiation indicating that there may be seasonal variation in 2n pollen production (Ortiz, 1997).

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