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Short communication

Irregular cytokinesis during microsporogenesis of psychotria myriantha Mull. Arg.

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ABSTRACT

The Rubiaceae family is composed of approximately 550 genera and 9000 species. In spite of its great number of species and importance, few cytological studies on the genus Psychotria are extant, whereas cytogenetic analyses deal with the number of chromosomes of a few species. This study describes for the first time the meiotic process during the microsporogenesis of P. myriantha. Analysis showed some irregularities during microsporogenesis and the most frequent is related to the cytokinesis process. Results show that 91.75% of microspores and 94.44% of pollen grains had normal behavior during the microsporogenesis process.

1. Introduction

The Rubiaceae family is composed of approximately 550 genera and 9000 species, mainly distributed in tropical regions (Souza and Lorenzi, 2005). Some species may be economical assets for the pharmaceutical and food industries; other species may be used as ornamental plants (Mendoza et al., 2004) and others still for feeding mammals (Gomes, 1996) and birds (Lorenzi, 1998).

The genus Psychotria, with approximately 1650 species (Hamilton, 1989) is the biggest genus of woody species of the family Rubiaceae (Davis et al., 2001).

In spite of its great number of species and importance, few cytological studies on the genus Psychotria are extant, whereas cytogenetic analyses deal with the number of chromosomes of a few species. There is a diversity of chromosome numbers in the genus ranging from 2n = 22, 32, 40, 44, 66, 88 and 132 (Bolkhovskikh et al., 1969; Goldblatt, 1981; Kiehn, 1986; Goldblatt and Johnson, 1994; Kiehn and Lorence, 1996; Corrêa and Forni-Martins, 2004; Corrêa et al., 2010).

Current analysis describes for the first time the meiotic process during the microsporogenesis of P. myriantha and focuses on an irregular aspect in the cytokinesis process during the same event.

2. Materials and methods

Botanic material was harvested at the Municipal Forest in Paranavaí (23º05´S & 52º27`W), in the state of Paraná, Brazil. P. myriantha´s reproductive parts were collected, exsiccated according to normal techniques (Fidalgo and Bononi, 1989) and deposited in the Herbarium of the Universidade Estadual de Maringá, Maringá PR Brazil. Young inflorescences were collected and fixed in ethanol/acetic acid (3:1 v/v) for 24 hours at room temperature to investigate their meiotic behavior. The material was washed and stored in ethanol 70% at 4°C. Slides were prepared by crushing and stained with acetic carmine 1.0%. Analyses were performed with an optic microscope. The same staining technique was employed to evaluate the viability of pollen grains by investigating color intensity and size of pollen grains. All abnormal features were registered and the most relevant were photographed with a Digital Celestron camera, only modifying brightness and contrast.

3. Results and Discussion

Analysis of the species P. myriantha reveals several bivalence-associated chromosomes in the phases of Prophase I (Figure 1a). It may be a species with a ploidy level higher than the tetraploid registered by Corrêa et al. (2010) in Psychotria carthagenensis. In current analysis, the number of chromosomes was not counted because of their small size and the overlaying of some chromosomes in diakinesis.

Table 1 shows irregularities during microsporogenesis and their frequency. The most frequent irregularity is related to the cytokinesis process. Cytokinesis is the cytoplasmic division, simultaneous or successive, that occurs during the plants´ microsporogenesis and which leads to the formation of the tetrad of haploid microspores (MAGNARD et al., 2001).

Cytokinesis is successive for most monocotyledons, whereas it is simultaneous for dicotyledons, such as the species of the genus Psychotria. Dyads are not formed at the end of meiosis I in simultaneous cytokinesis since two cytoplasmic divisions occur at the end of meiosis II which form a microspore tetrad.

However, irregular cytokinesis is reported in 21.83% of Prophases II and at different frequencies (Table 1) in other phases of meiosis II (Figure 1d - g), similar to successive cytokinesis where cytokinesis occurs at the end of the first division and leads towards the formation of dyads.

Meiosis is characterized by a set of mechanical and chemical events whose product is the formation of four haploid microspores. Golubovskaya (1989) reports that events are genetically controlled wherein are extant the genes that promote cytokinesis. The latter were described by Beadle (1932) in his investigations on maize.

Gene tardy asynchronous meiosis (tam) was mapped from chromosome 1 of Arabidopsis thaliana, albeit still not well known. Protein tam possibly regulates the cell cycle progression and may be related with G2/M transition. It may be the cause of the synchronism of the division during male meiosis (MAGNARD et al., 2001).

Consiglio et al. (2003) report that gene tam mutation alters the normal rhythm of the cell division. In this mutant, the generally simultaneous cytokinesis behaves as if it were successive. Mutation in the gene tam delays the cell division process in male meiosis. However, cytokinesis is not significantly altered and results in the formation of normal tetrads.

Magnard et al. (2001) relate changes in the plants´ tetrads by temperature since their investigations showed that plants with gene tam alterations grown at 22°C produced a 5% to 90% variation of abnormal tetrads. Further, 100% of tetrads produced abnormalities in plants grown in hothouses at 27°C.

In P. myriantha, high temperature may have also contributed towards the formation of dyads (Figure 1h), triads (Figure 1i) and polyads (Figure 1j). In fact, mean temperature in October 2012, when the collection of the species under analysis was undertaken, varied between 18.3ºC and 29.5ºC, with highest temperature at 38.9ºC during the period (SMC/IAPAR, 2012).

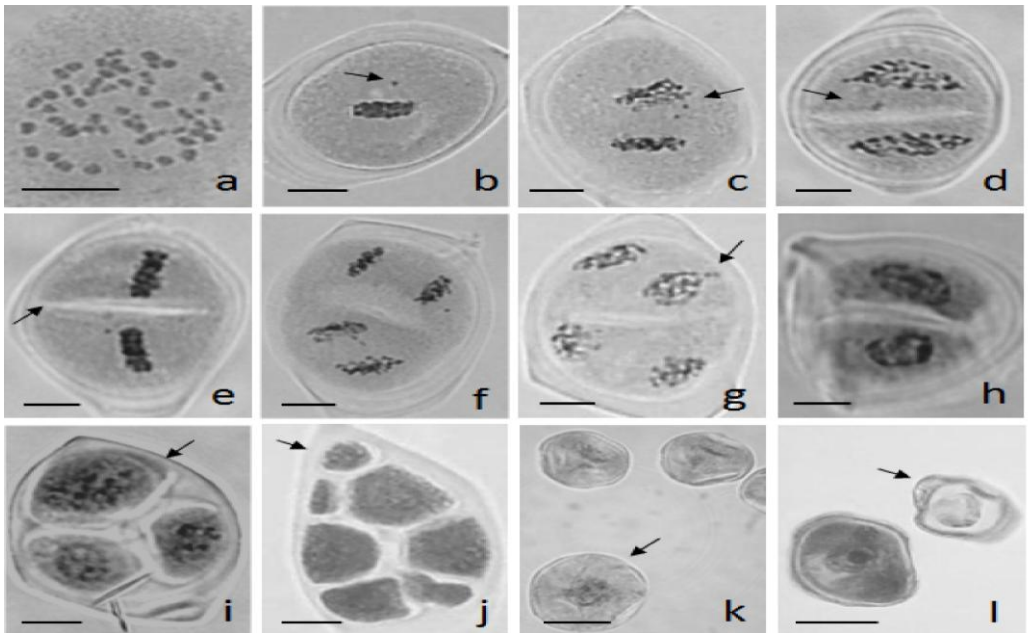

Fig. 1. Aspects of microsporogenesis in P. myriantha. a – Diakinesis; b - metaphase I with precocious chromosome migration (arrow); c - Anaphase I with laggard chromosomes (arrow); d - prophase II with micronuclei (arrow) and irregular cytokinesis; e - metaphase II with irregular cytokinesis (arrow); f - anaphase II with laggard chromosome and irregular cytokinesis; g - telophase II with micronucleus (arrow) and irregular cytokinesis; h - dyad; l - triad; j - polyad with microcytes (arrow); k - normal and 2n (unreduced) microspores (arrow); p - normal and unbalanced pollen grain (arrow). Bar = 10µm.

Table 1
Abnormalities observed during microsporogenesis in P. myriantha.

Phase	No. of cells analyzed	No. of normal cells (%)	No. of abnormal cells (%)	Abnormalities
Metaphase I	164	145 (88.42%)	19 (11.58%)	Precocious migration -19 (11.58%)
Anaphase I	118	108 (89.53%)	10 (8.47%)	Laggards chromosome -10 (8.47%)
Telophase I	111	109 (98.20%)	2 (1.80%)	Micronuclei -1 (0.90%) Irregular cytokinesis -1 (0.90%)
Prophase II	142	110 (77.47%)	32 (22.53%)	Micronuclei -1 (0.70%) Irregular cytokinesis -31 (21.83%)
Metaphase II	123	107 (86.99%)	16 (13.01%)	Precocious migration -6 (4.88%) Irregular cytokinesis - 10 (8.13%)
Anaphase II	115	108 (93.92%)	7 (6.08%)	Laggards chromosome -1 (0.86%) Irregular cytokinesis - 6 (5.22%)
Telophase II	146	128 (87.68%)	18 (12.32%)	Micronuclei - 1 (0.68%) Irregular cytokinesis - 15 (10.27%) Nuclear restitution -2 (1.37%)
Tetrad	236	217 (91.95%)	19 (8.05%)	With microcytes - 5 (2.12%) Dyad - 3 (1.27%) Triad - 8 (3.39%) Polyad - 3 (1.27%)
Microspores	216	196 (90.75%)	20 (9.25%)	Unbalanced – 14 (6.47%) 2n microspore - 6 (2.94%)
Pollen grain	234	221 (94.45%)	13 (5.55%)	Unbalanced – 7 (2.99%) 2n pollen grain – 6 (2.56%)

However, other types of non-regularity, such as precocious chromosomes migration, Laggard chromosomes, micronucleus and microcytes, may have contributed towards the formation of abnormal tetrads in P. myriantha.

Chromosomes in precocious migration (Figure 1b) may be related to chiasmata´s early terminalization process in Metaphase I. In fact, chiasmata cause the maintenance of bivalent chromosomes so that perfect segregation may occur in Anaphase I. Laggard chromosomes in Anaphase I (Figure 1c) may have been caused by the chiasmata´s late terminalization and by late rupture of the centromere in Anaphase II.

Chromosomes that migrate early to the poles may either delay and may not be included in the telophase nuclei by forming micronuclei, or they may be eliminated as microcytes and cause a gene imbalance which affects the fertility of the pollen grain (ADAMOWSKI et al., 2000).

Irregularities, such as chromosomes in precocious migration and laggards, have been frequently described in different species of several genera, such as in the Paspalum maritimum (ADAMOWSK et al., 2000), Brachiaria brizantha (RISSO-PASCOTTO et al.,Micronuclei of P. myriantha formed during meiosis I end meiosis II and were eliminated in one or more microcytes (Figure 1j), causing the formation of microspores and imbalanced pollen grains.

Viability of gametes was based on color and size of microsporeins. Microspores with intense color and size between 10 and 12µm were classified as normal; microspores that measured between 18 and 22µm, with a similar color as that of the normal ones, were classified as 2n. Microspores with shrunk cytoplasm and only slightly stained were classified as imbalanced. The same criteria were used to classify pollen grains (Table 1).

In spite of certain irregularities in current analysis, meiosis did not undergo relevant alterations. Similar behavior was also reported in studies on Arabidopsis thaliana (CONSIGLIO et al., 2003). Results show that, at the end of P. myriantha meiosis, 91.75% of microspores and 94.44% of pollen grains had normal behavior during the microsporogenesis process.

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