


Artigos

Cytogenetic study and pollen viability of *Diatenopteryx sorbifolia* Radlk.

Estudo citogenético e viabilidade do pólen de *Diatenopteryx sorbifolia* Radlk.

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ABSTRACT

The number of chromosomes and the meiotic characterization are important to studies involving genetic variability, germplasm and biodiversity. However, native forest species have not been sufficiently analyzed from a cytotaxonomic point of view. Therefore, the number of chromosomes, the meiotic behavior and the pollen viability of the *Diatenopteryx sorbifolia* Radlk were determined from five different accessions located in the southern region of Brazil. The species was considered diploid, $2n = 30$ ($x = 15$), with regular meiotic behavior in phase I and absent in phase II, suggesting the presence of a genetic abnormality. Pollen grains infertility was between 57 and 67%, which may be associated to the formation of a low number of seeds in *Diatenopteryx sorbifolia*, probably due to the irregular behavior of the microsporogenesis.

Keywords: Forest seeds; Genetic characterization; Reproduction; Sapindaceae

RESUMO

O número de cromossomos e a caracterização meiótica são importantes para estudos envolvendo variabilidade genética, germoplasma e biodiversidade. Entretanto, espécies florestais nativas não são suficientemente analisadas do ponto de vista citotaxonômico. Dessa forma, o número de cromossomos, o comportamento meiótico e a viabilidade polínica de *Diatenopteryx sorbifolia* Radlk foram determinados a partir de cinco acessos diferentes localizados na região sul do Brasil. A espécie foi considerada diplóide, $2n = 30$ ($x = 15$), com comportamento meiótico regular na fase I e ausente na fase II, sugerindo a presença de uma anormalidade genética. A infertilidade dos grãos de pólen ficou entre 57 e 67%, o que pode estar associado à formação de um baixo número de sementes em *Diatenopteryx sorbifolia*, provavelmente em decorrência do comportamento irregular na microsporogênese.

Palavras-chave: Sementes florestais; Caracterização genética; Reprodução; Sapindaceae

1 INTRODUCTION

The Sapindaceae, considered a cosmopolitan family, covers about 140 genera and 1,800 species (COULLERI; FERRUCCI, 2012) of tropical, subtropical, and rarely temperate occurrence (JUDD et al., 2009). In Brazil, there are 27 genera and 419 species (SOMNER et al., 2014), and among them, the *Diatenopteryx sorbifolia* Radlk., popularly known as maria-preta, arboreal, reaching height between 10 and 35 m (LORENZI, 2002).

In addition to its ornamental and timber qualities, *Diatenopteryx sorbifolia* is recommended for altered recovering ecosystems, mainly riparian forests, where it endures flooding (LORENZI, 2002; CARVALHO, 2003). In the Atlantic Forest, Brazil, this species is found in Seasonal, Mixed and Dense Ombrophylous Forest (CARVALHO, 2003), and it is distributed in different vegetation formations, with unquestionable ecological importance. However, due to deforestation over the years, there are few surviving examples of the species in southern Brazil (FELIPPI et al., 2013).

The *Diatenopteryx sorbifolia* has phenological asynchrony and a different number of plants in reproductive phenophase depending on the year (FELIPPI et al., 2013). It also presents a low number of fruits containing seeds, making it difficult to form homogeneous lots (FELIPPI et al., 2013), which may have implications for species

regeneration in natural environment, seed analysis and seedling production. Thus, we suggest studies focusing on embryogenesis, formation and development of *Diatenopteryx sorbifolia* seeds, as well as on genetic information that may contribute to understand the ecology of this species or to distinguish its reproductive behavior.

Considering that the genotype manifestation of the individual is the result of the contribution brought by gametes in zygote formation, a higher rate of viability and pollen germinability results in greater possibility of the production of distinct combinations between alleles and genetic variability (AKORODA, 1983). Thus, the plant fertility can be measured initially by the analysis of its meiotic process since the production of viable pollen grains is directly associated with the regularity of the process. Although many species produce a high percentage of viable pollen, not all of them are used in fertilization effectively, since they can be lost when carried by the wind or insects. The higher the pollen viability is, the greater the fertilization success is (MARTINS *et al.*, 2010).

Abnormalities affecting the plant fertility have been described showing that meiotic regularity is a basic requirement for viable gamete productions (PAGLIARINI, 2000). The determination of the chromosome number, the characterization of the meiotic behavior and the pollen viability could aid in a reproductive study of *Diatenopteryx sorbifolia*. It is noteworthy that only 150 species belonging to 42 genera of the Sapindaceae family had their chromosome number described (URDAMPILLETA *et al.*, 2013) and the total amount of studies involving native species in which arboreal habit predominates is a limiting factor.

In order to gather key information about the *Diatenopteryx sorbifolia* species, we recorded the chromosome numbers, male gamete formations (microsporogenesis) and pollen fertility in Rio Grande do Sul and Paraná accessions.

2 MATERIALS AND METHODS

2.1 Collection of the botanical material

The botanical material was collected during the reproductive phenophases, from five mother trees (genotypes) (G1, G2, G3, G4 and G5). The first four being present in remnants of the Deciduous Seasonal Forest in Frederico Westphalen town (27° 21' 33" S; 53° 23' 40" W), Rio Grande do Sul state, and the fifth from the Mixed Ombrophilous Forest located in Iguaçu Falls (25° 26' 27"S, 52° 55' 17" W), Paraná state, Brazil.

2.2 Meiotic analysis

During the flowering phenophase, pre-anthesis floral buds were collected, between October 2013 and November 2015, and fixed in Carnoy (ethanol-acetic acid 3:1), remaining for 24 hours at room temperature, then transferred to a 70% alcohol solution and finally kept under refrigeration (6 to 10°C) until analysis. When preparing slides, the anthers were crushed and the myocytes stained with 1% of propionic carmine, according to Guerra and Souza (2002). For the meiotic behavior analysis were analyzed from metaphase phase I until microspores tetrad, counting at least 500 microsporocytes per accession.

2.3 Pollen viability

For the counting and analysis of the pollen grains we used at least 1,000 cells per accession, each cell stained with 1% of lugol dye. The method of staining with lugol is based on a chemical reaction between the iodine and the starch molecule, making the pollen grains brown and non-viable, with a light yellow to transparent staining (PAGLIARIANI; POZZOBON, 2004). By doing that, the grains that presented weak or absent staining, reduced or absent protoplasm were considered unfeasible, and those with intact exine, well-stained protoplasm with homogeneous distribution were considered feasible.

2.4 Mitotic analysis

During the fruiting period, ripe fruits were collected for the later seed processing, and then germinated in acrylic boxes with lid, containing vermiculite of sterilized, moistened medium particle size. When the primary roots had about 2 cm, they were collected and pretreated with 8-hydroxyquinoline solution (8HQ) at 0.002M for 24h at 6° to 10°C, to obtain a higher number of cells in metaphase. Finally, the roots were washed and fixed in Carnoy (ethanol-acetic acid 3: 1), remaining for 24 hours at room temperature, and then stored in alcohol 70% (6 a 10°C).

Schiff staining technique was used for the preparation of the slides. The roots were washed in distilled water, hydrolyzed in HCl 1N at 60°C for 10 minutes and then washed again and placed in the dye, remaining immersed for 30 minutes. The root end was crushed with a drop of 1% propionic carmine. Five to ten metaphases per genotype were counted, and the metaphases that presented well-condensed, visible, spread and non-overlapping chromosomes on the slide were considered for characterization and photographic registration. The germination of the seeds was done for all the genotypes. However, only the G5 was possible to make the analyzes. The remaining phase M cells were not observed.

2.5 Slide analysis in microscopy

Both meiotic and mitotic analyzes were performed on an Olympus X31TM image capture microscope, where the most representative phases were captured with the aid of the Pixelview station v5 23 TV software and scanned by Corel Photo-Paint X6. Cells counted: empty cells, where no genetic material was found; cells with incrustations, which crystals prevented the genetic material visualization and the cells with abnormalities, which were considered when any abnormality was observed in the segregation of the chromosomes.

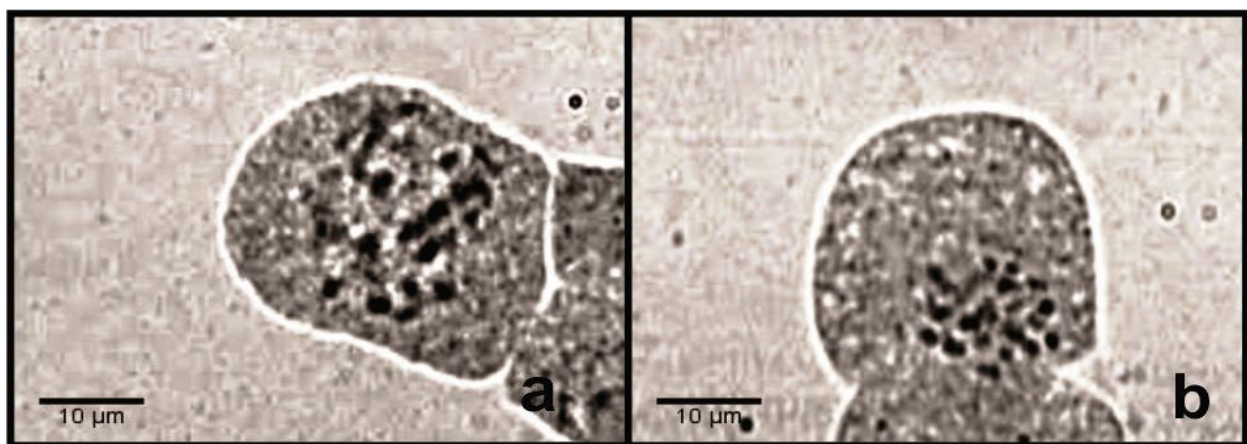
2.6 Data analysis

Data were submitted to descriptive statistics, and frequencies submitted to the analysis of association in chi-square test contingency table using the Past software (HAMMER; HARPER; RYAN, 2001).

3 RESULTS AND DISCUSSION

Adiploid characteristic was observed in *Diatenopteryx sorbifolia*, with chromosome number of $2n = 30$ ($x=15$), (Figure 1a and 1b), number described by Ferrucci and Solís Neffa (1997). Genus *Diatenopteryx* shows $2n = 30$ chromosomes (FERRUCCI; SOLÍS NEFFA, 1997), being that the basic numbers $x = 14, 15$ and 16 are present in 90% of the genera, probably $2n = 28, 30$ or 32 the family's ancestral chromosome number (FERRUCCI, 2000; SOLÍS NEFFA; FERRUCCI, 2001; URDAMPILLETA *et al.*, 2013).

Figure 1 – a and b Somatic cells of *Diatenopteryx sorbifolia* Radlk containing $2n = 30$ chromosomes in metaphase



Source: Authors (2020)

Polyploidy is one of the main mechanisms of the karyotype evolution in angiosperms, making possible a fast genomic restructuring that favors the diploidization of new species (LEITCH; BENNETT, 2004; SCHIFINO-WITTMANN, 2004). Some authors

suggest that chromosome numbers in most angiosperms were derived from polyploidy or dysploidy/aneuploidy ($x = 8$) (SCHIFINO-WITTMANN, 2004). However, polyploidy does not appear to be an important mechanism in the recent evolution of Sapindaceae, with polyploidy variations cited in species of the genus *Acer*, through phylogenetic analysis using DNA sequences, Grimm *et al.* (2006) recognized several tetra-, hexa- and octaploid polyploids.

In species of *Urvillea* tetraploid ($2n = 4x = 44$) and octaploid ($2n = 8x = 88$) were found (FERRUCCI, 1981; URDAMPILLETA *et al.*, 2006). Polyploidy was described in the *Paullinia*, where for *Paullinia cupana* var. *sorbilis*, guarana, with $2n = 210$ chromosomes (FREITAS *et al.*, 2007). In *Melicoccus* P. Browne (Sapindaceae - Melicocceae) with their two species studied cytologically, there are two levels of ploidy, $2n = 2x = 32$ and $2n = 6x = 96$, representing the highest number of chromosomes in Sapindaceae (FERRUCCI; SOLÍS NEFFA, 1997).

According to Guerra (1988), a clear and precise characterization of the karyotype of a species is of fundamental importance when one wants to compare cytogenetically different species, or to examine the variation between individuals of same species.

In the meiotic analyzes carried out on the five *Diatenopteryx sorbifolia* genotypes, differences were observed in the meiotic behavior among the matrices (Table 1). It was verified that there is a significant association between the evaluated parameters and the genotypes. Thus, the frequency of empty cells, incrustations or abnormalities depended on the genotype. For G5 genotype, 2513 meiocytes were analyzed. They showed a meiotic regular behavior in phase I of meiosis. Phase II of meiosis was not observed, but the presence of meiocytes with total absence of genetic material was observed (Figure 2a), resulting in more than 60% of non-viable pollen grains (Figure 3a), possibly affecting the species rate of fertilization.

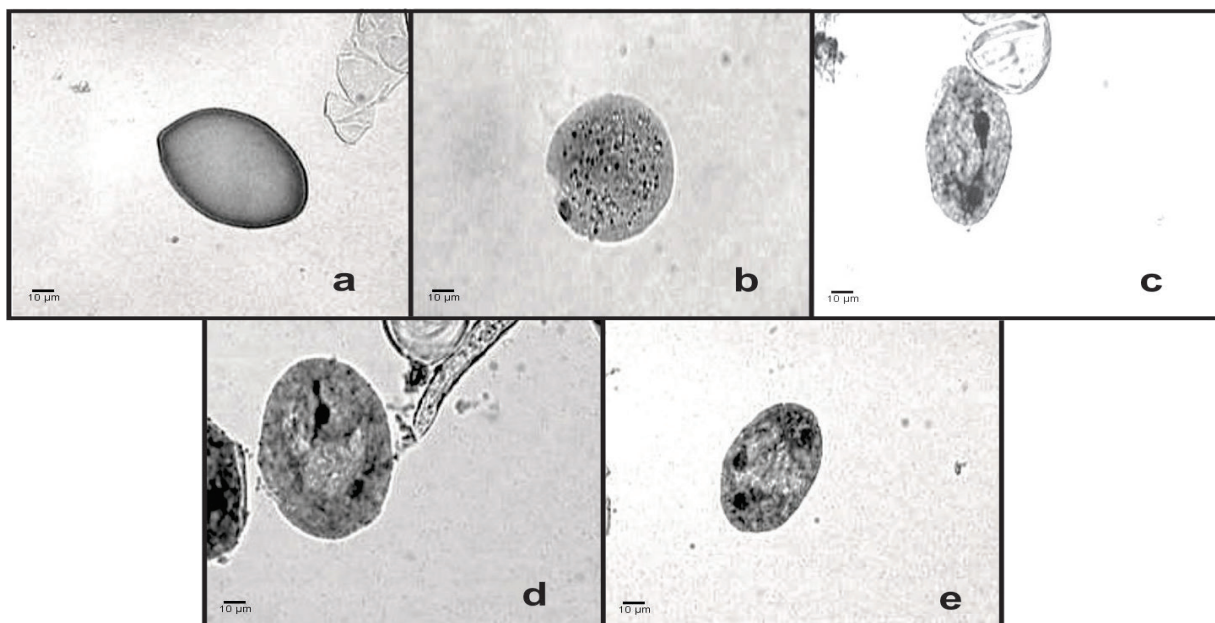
Table 1 – Genotype, numbers of chromosomes (2n) and meiocytes analyzed, frequency (%) of empty cells, with incrustations and abnormalities in *Diatenopteryx sorbifolia* Radlk

Genotype	N. Chromosomes (2n)	N. meiocity analyzed	Cell frequency (%)		
			Empty	With incrustations	With abnormalities
G1		1060	13.7*	27.2*	25.8*
G2		1050	4.8	19.0	72.1
G3		1323	4.1	21.1	63.0
G4		1170	7.6	19.6	71.0
G5	30	2513	10.1	19.3	50.8
Mean	-	1423.2	8.0	21.2	56.5
Total	-	7116	-	-	-

Source: Authors (2020)

In where: * = Significant association ($p < 0.01$) in contingency analysis with chi-square test.

Figure 2 – Meiotic characterization of *Diatenopteryx sorbifolia* Radlk. a - empty cell (G5). b - cell with incrustations (G2). c - anaphase I with adherence (G1). d - telophase I with micronucleus (G1). e – triad (G1)



Source: Authors (2020)

In where: *Arrow in letter d indicates the micronucleus.

Figure 3 – *Diatenopteryx sorbifolia* Radlk. a - infertile pollen grains. b - infertile pollen grain (with little protoplasm). c - grain of fertile pollen



Source: Authors (2020)

Except for G1 genotype (Table 1), the others presented similar meiotic behavior. In G5, meiocytes were observed with total absence of genetic material. Thus, although matrices from different locations and influenced by different environmental and climatic factors, their meiotic approach are very similar, indicating the presence of genetic abnormalities.

G1 genotype behavior was regular in two phases of the meiosis (Table 1), with 25% of typical abnormalities - such as chromosomal adhesions in anaphase I (Figure 2c), telophase I with micronucleus (Figure 2d) and triads (Figure 2e). It is important to emphasize that meiosis is characterized by the occurrence of a series of mechanical and biochemical events that result in the formation of four haploid microspores and mutations in the genes that control these events cause abnormalities and may compromise the fertility of many species, causing meiotic modifications such as chromosomal adhesion, fusions, cytokinesis, irregular chromosomal segregation and partial or total absence of meiosis (BOLDRINI *et al.*, 2011). Thus, gametes carrying abnormalities lose competitiveness with normal gametes because they cause decrease in fruit formation with or without seeds (PAGLIARINI, 2001).

The presence of incrustation in cells cytoplasm (Figure 2b), ranging from 19 to 27.2 (Table 1), made it difficult to prepare meiocytes and to observe the genetic

material. These incrustations possibly hinder the normal course of microsporogenesis. Therefore, it is necessary to verify their origin, composition and function, since they are observed in other forest species.

For the pollen grains (Table 2), the analysis revealed no more than 57% of non-viability, and in G5 genotype 67% were found containing little or no protoplasm and transparent staining (Figure 3a, 3b). Therefore, fertile pollen (Figure 3c) ranged from 32.4% (G5) to 43% (G3).

Table 2 – Viability* of *Diatenopteryx sorbifolia* Radlk pollen grains expressed as percentage (%)

Genotype	N. pollen analyzed	% of pollen	
		Fertile	Infertile
G1	1512	35,2*	64,8
G2	1063	36,7	63,3
G3	1715	43	57
G4	1307	40,5	59,5
G5	2013	32,4	67,6
Mean	1522	37,5	62,5
Total	7610	-	-

Source: Authors (2020)

In where: * = Significant association ($p < 0.01$) in contingency analysis with chi-square test.

The high amount of infertile pollen in *Diatenopteryx sorbifolia* accessions is probably due to the irregular behavior of the microsporogenesis. This fact contributes to the low number of seeds produced, even when with fruitage fully formed. Singh (2002) points out the high percentage of pollen grain sterility with the occurrence of meiotic irregularities, which consequently lead to the production of pollen with decreased viability.

However, fertile pollen frequency had a significant association with the genotypes evaluated (Table 2). This indicates that there is a genetic or an environmental variation for this characteristic. The observed variation may not only diverge between individuals

of the same species, but also between samples of the same individual. According to Shivanna and Johri (1992), the blossom period, environmental changes and genotypic differences may contribute to such variance. For Pagliarini and Pozzobon (2004), the primary reason for viability decrease of pollen stored for short or long periods is related to the inactivation of enzymes and metabolic substrates which are essential for germination. Accumulations of secondary metabolic products, such as organic acids, may also inhibit germination. Genetic factors, such as chromosomal unbalances, caused by meiotic abnormalities, also affect the viability of pollen grains. In this case, the unviability could be observed in pollen grains just released from the anther. Thus, through colorimetric analysis it is possible to estimate only the pollen viability. But pollen fertility is determined by the germination and the pollen tube growth or by fluorochromatic tests (HISTER; TEDESCO, 2016).

It is important to point out the need for further research related to cytogenetics and reproductive systems of native forest species such as *Diatenopteryx sorbifolia*, which are still little known, could contribute to seed production. According to Biondo, Miotto and Schifino-Wittmann (2005) collections of botanical material from various plant formations contribute to more comprehensive conclusions. Therefore, the importance and demand for studies of basic cytogenetic characterization in native forest species in southern Brazil.

4 CONCLUSION

A diploid characteristic was observed in the *Diatenopteryx sorbifolia* Radlk, with chromosome number of $2n = 30$, regular meiotic behavior present in phase I and absent in phase II for 80% of the accessions, ending up with an amount of infertile pollen between 57 and 67%. This may have contributed to the low number of seeds formed as observed in previous studies.

ACKNOWLEDGEMENTS

The authors would like to thank “Fundação Araucária” for funding the research project.

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How to quote this article

Filippi, M.; Boldrini, K. R.; Agostinho, K. F.; Corrêa, B. J. S.; Donazzolo, J. Cytogenetic study and pollen viability of *Diatenopteryx sorbifolia* Radlk. *Ciência Florestal*, Santa Maria, v. 32, n. 1, p. 233-246 2022. DOI 10.5902/1980509848072. Available from: <https://doi.org/10.5902/1980509848072>.