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Research Article

Viability and meiotic index of *Ochroma pyramidale* from the Brazilian Amazon[1]

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ABSTRACT

Ochroma pyramidale is a neotropical tree species native to Central and South America, occurring naturally in the Brazilian Amazon and cultivated in the Mato Grosso state, Brazil, where it is used for timber production and ecological restoration. This study aimed to investigate the pollen viability and meiotic behaviour of *O. pyramidale* (Cav. ex Lam.) Urb., factors that directly influence the species' reproductive success. Flower buds at various developmental stages were collected from six individuals to assess the pollen grain morphology, pollen viability and meiotic index. The pollen viability (mean values of 96.16 and 97.72 %, using acetocarmine and the Alexander's stain, respectively) and meiotic index (average of 96.53 %) were high across all individuals, presenting regularity and stability in the meiosis process.

KEYWORDS: Balsa wood, pollen grains, meiotic stability.

RESUMO

Viabilidade e índice meiótico de *Ochroma pyramidale* da Amazônia brasileira

Ochroma pyramidale é uma espécie arbórea neotropical nativa das Américas Central e do Sul, encontrada na Amazônia brasileira e cultivada no estado de Mato Grosso, Brasil, visando à produção de madeira e recuperação de áreas degradadas. Objetivou-se estudar a viabilidade e o comportamento meiótico do grão de pólen que interferem na reprodução de *O. pyramidale* (Cav. ex Lam. Urb., Malvaceae). Botões florais em diferentes estágios de desenvolvimento foram coletados de seis indivíduos para estimar a morfologia do grão de pólen, viabilidade polínica e o índice meiótico. A viabilidade polínica (médias de 96,16 e 97,72 %, utilizando-se acetocarmina e coloração de Alexander, respectivamente) e o índice meiótico (média de 96,53 %) foram altos para todos os indivíduos avaliados, apresentando regularidade e establidade no processo de meiose.

PALAVRAS-CHAVE: Pau-de-balsa, grãos de pólen, estabilidade meiótica.

INTRODUCTION

Ochroma pyramidale (Cav. ex Lam.) Urb. (synonym *Ochroma lagopus*), commonly known as balsa wood, belongs to the Malvaceae family (Carvalho-Sobrinho 2025). It is a widely distributed neotropical tree species, found in western India and throughout tropical America, occurring naturally in southern Mexico, Central America, the Antilles and South America (Morante-Alarcón et al. 2017). In Brazil, its distribution includes the states of Acre, Amazonas, Pará and Roraima (Carvalho 2010).

Ochroma pyramidale exhibits promising characteristics for cultivation and production in Brazil, being a native, pioneer and fast-growing species (Bizuti et al. 2016, Gomes & Reis 2018, Lima Júnior et al. 2019). Research on the ecology and genetics of tropical tree species provides essential information for conservation and breeding efforts, supporting reproduction and the maintenance of genetic diversity in managed populations (Maués & Oliveira 2010), including for the cultivation of *O. pyramidale.*

The study and characterization of pollen grains are critical for various subjects, particularly

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taxonomy, genetic breeding, phylogeny and paleobotany (Nunes et al. 2012). Morphological traits such as shape, size, aperture number and exine ornamentation are considered taxonomically significant, as they remain consistent within species (Blackmore 2007, Talledo et al. 2019). However, a literature review revealed only one study (Carreira et al. 1995) describing the pollen morphology of *O. pyramidale*, under the synonym *O. lagopus* Sw.

Plant fertility is closely linked to meiotic regularity, which affects pollen grain formation (Pereira et al. 2017). The meiotic index, calculated based on post-meiotic products (e.g., tetrads, triads, dyads, monads), serves as a complementary indicator of meiotic regularity (Oliveira & Pierre 2018).

Pollen viability is a key measure of male fertility. High viability rates reflect regular meiosis and a high meiotic index, indicating genotype potential and influencing fertilization success (Cabral et al. 2013, Zambrano et al. 2019).

Cytochemical analyses of pollen grains are commonly used to detect the presence of reserve substances. Identifying specific storage compounds in pollen provides valuable insights into ecological aspects related to pollination mechanisms (Dettke & Santos 2011, Oliveira & Pierre 2018).

Despite its ecological and economic relevance, research on the reproductive biology of *O. pyramidale* - including pollen germination, viability and storage - remains scarce (Zambrano et al. 2019). In this context, the present study aimed to evaluate reproductive aspects of *O. pyramidale* to support the planning of breeding programs and the selection of genotypes with high fertilization rates, contributing to the conservation of the species.

MATERIAL AND METHODS

Flower buds of *Ochroma pyramidale* at various developmental stages were collected in February 2019 from six individuals in three municipalities located in the northern region of the Mato Grosso state, within the Brazilian Amazon biome: Sinop (SP01, SP02 and SP03), Itaúba (IT04 and IT05) and Alta Floresta (AF06). The material was fixed in a solution of absolute ethanol and glacial acetic acid (3:1 v/v) at the time of collection, transferred to 70 % ethanol after 24 hours, and stored under refrigeration at 20 ºC. All analyses were conducted at the Universidade do Estado de Mato Grosso Carlos Alberto Reyes Maldonado, in Alta Floresta, Mato Grosso state, Brazil.

Pollen morphology was analyzed using the acetolysis method proposed by Erdtman (1943). Two slides were prepared per sample, each containing two drops of the material in glycerin, and examined under an optical microscope at 400x magnification. Pollen grains were photographed with a Biocam digital camera coupled to a microscope and analyzed using the TsView 7 image capture system and the Anati Quanti 2® UFV software (Aguiar et al. 2007).

Measurements were taken from 25 pollen grains in equatorial view and 25 in polar view. Measured parameters included polar diameter (polar axis) and equatorial diameter in equatorial view (with the grain oriented perpendicular to the polar axis), equatorial diameter in polar view (with the polar area facing the observer), exine layers (sexine and nexine) thickness, and aperture diameters.

The shape of the apertures was determined by the ratio between their two diameters. Pollen grain analyses followed the methodologies of Erdtman (1952) and Barth (1964). The classification of pollen size was based on the length of the longest axis. Pollen shape and symmetry were assessed using the polar-to-equatorial axis ratio in equatorial view. Grains were also classified according to the polar area index (Barth & Melhem 1988), which is calculated from the distance between two adjacent apertures relative to the maximum width of the pollen grain in polar view. For each parameter, the mean, standard deviation, coefficient of variation and confidence intervals were estimated using the Genes software (Cruz 2013).

The meiotic index was estimated for all six individuals of *O. pyramidale*. Anthers were separated and gently macerated in a drop of 2 % acetic carmine solution (Radford et al. 1974). Eight slides were prepared per individual, and 300 post-meiotic products were analyzed per slide, totaling 2,400 observations. Tetrads with four equally sized cells were considered normal; deviations such as monads, dyads, triads or polyads were considered abnormal. The meiotic index (MI) was calculated using the formula proposed by Love (1951): MI = [(number of normal post-meiotic products)/(total number of abnormal and normal post-meiotic products)] × 100.

Descriptive statistics (mean, standard deviation and coefficient of variation) were calculated using the Genes software (Cruz 2013). The data were also subjected to analysis of variance using a generalized linear model with binomial distribution, implemented in the R software (R Core Team 2016).

Pollen viability was estimated for the six individuals using colorimetric staining with 2 % acetic carmine (Radford et al. 1974) and the Alexander's stain (Alexander 1969). Anthers were separated and gently macerated on microscope slides in a drop of the respective dye. Eight slides were prepared per individual, with 300 pollen grains examined per slide, totaling 2,400 grains per dye. Pollen viability was expressed as the percentage of viable pollen grains, calculated by the formula: Pollen viability (%) = [(number of viable grains)/(number of counted grains)] x 100.

The data were analyzed by analysis of variance using a generalized linear model appropriate for binomial distribution and a chi-square test, both performed using the R software (R Core Team 2016).

For cytochemical analysis, anthers from pre-anthesis flower buds were macerated on microscope slides and stained with the Lugol's solution (Baker & Baker 1979) to detect starch, and Sudan IV (Dafni 1992) to detect lipids. Eight slides were prepared per stain, with 300 pollen grains analyzed per slide, totaling 2,400 grains per dye. For each stain, the percentage of positively stained pollen grains - those indicating the presence of starch or lipids - was calculated to determine the main reserve substance in the pollen.

RESULTS AND DISCUSSION

The measurements of the pollen grains of *Ochroma pyramidale* are presented in Table 1. The pollen grains are 3-porate (Figure 1A), with equatorial diameters ranging from 188.5 to 225.89 µm and polar axes ranging from 157.9 to 183.2 µm. Pollen grains with a major axis exceeding 200 µm are classified as giant, which applies to the equatorial diameter (EV) of *O. pyramidale*.

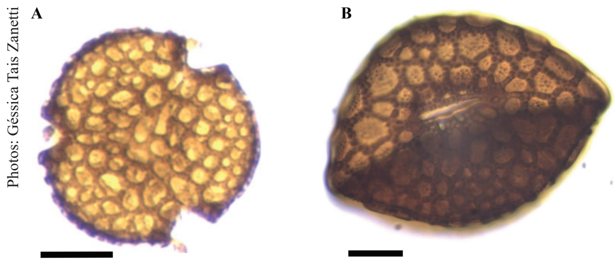

Figure 1. Pollen morphology of *Ochroma pyramidale*. A) detail of the reticulate ornamentation of the exine (polar view); B) detail of the pollen aperture (equatorial view). Scale bar = 50 µm.

The exine surface of *O. pyramidale* pollen shows a reticulate ornamentation with polygonal lumens (Figure 1A), and the grains have a sub-oblate shape with radial/isopolar symmetry. The aperture, determined by the ratio between its two diameters, was less than 2, classifying it as a pore (Figure 1B). The polar area index indicated a large polar area. The average exine thickness was 8.06 µm, primarily composed of the sexine layer (4.65 µm).

The pollen grains of *O. pyramidale* are 3-porate, with a sub-oblate shape and radial/isopolar symmetry. These results are consistent with those reported by Carreira et al. (1995) and Salgado-Labouriau's (1973) for the Bombacaceae family. The measurements for the polar axis, equatorial axis, polar axis/equatorial diameter ratio, nexine and sexine were higher than those reported by Carreira et al. (1995) for the synonymous species *Ochroma lagopus* Sw.

The cross-linked ornamentation observed in this study agrees with the findings of Carreira

Table 1. Measurements of pollen from *Ochroma pyramidale* subjected to the acetolysis method.

Measurement	Min-Max (µm)	x ± sx (µm)	CI 95% (µm)	CV (%)
Equatorial diameter (equatorial view)	188.51-225.79	207.33 ± 9.16	203.26-210.54	4.42
Polar axis (equatorial view)	157.98-183.2	168.31 ± 7.68	171.39-164.30	4.56
Equatorial diameter (polar view)	224.75-179.45	194.77 ± 11.82	189.52-199.5	6.06
Nexine	2.17-4.74	3.40 ± 0.65	3.11-3.06	19.27
Sexine	3.5-6.47	4.65 ± 0.72	4.33-4.94	15.57
Exine	5.67-11.21	8.06 ± 1.24	7.50-8.55	15.40
Polar axis/equatorial diameter ratio	0.75-0.91	0.81 ± 0.04	0.79-0.83	5.91
Polar area index	0.56-0.71	0.66 ± 0.03	0.65-0.67	4.58

x: mean; sx: standard deviation; CI 95 %: confidence interval; CV: coefficient of variation.

et al. (1995) and Gutiérrez & Mosquera (2016). Exine ornamentation is a key morphological feature in pollen identification and species delimitation (Salgado-Labouriau 1973, Blackmore 2007). According to Reis & Paludzyszyn Filho (2011), *O. pyramidale* is pollinated by bats, what explains the presence of the cross-linked exine ornamentation.

As highlighted by Zambrano et al. (2019), understanding pollen biology in *O. pyramidale* is crucial for planning controlled breeding in conservation and genetic breeding programs. These findings provide valuable information for palynological studies and taxonomic identification of the species.

The post-meiotic products and meiotic indices for each individual are shown in Table 2. Post-meiotic products were observed in flower buds measuring 38.05-50.46 mm in length. The majority of meiocytes (97.72 %) were in the tetrad stage (Figure 2A), indicating a low level of meiotic irregularity. Triads were the most commonly observed abnormality (Figure 2B).

All six individuals of *O. pyramidale* exhibited high meiotic indices (> 91 %). Figure 3 presents the boxplots for meiotic index. The AF06 genotype had a lower mean meiotic index (91.92 %), if compared to the others, as well as the highest variability, and a standard deviation of 3.68. The remaining genotypes had means close to 100 %, with low variation and standard deviations ranging from 0.49 to 1.33.

Photos: Géssica Tais Zanetti

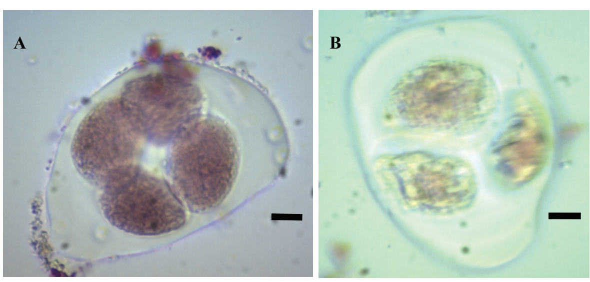

Figure 2. Post-meiotic products observed in *Ochroma pyramidale*. A) normal tetrad; B) triad. Scale bar = 20 μm.

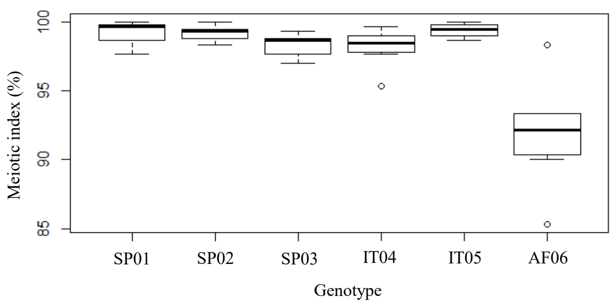

Figure 3. Mean percentage and standard deviation of the meiotic index found for each individual of *Ochroma pyramidale*.

The predominance of normal tetrads in *O. pyramidale* indicates a high meiotic stability, with a mean meiotic index of 97.72 %. This supports the assertion by Love (1951) that plant species with meiotic index values above 90 % exhibit high meiotic stability, which contributes to proper tetrad formation. Although abnormalities such as polyads, triads, dyads and monads were observed, they occurred at low frequencies. According to Wang et al. (2010) and Zhang et al. (2017), such abnormalities result from irregular chromosome pairing, spindle formation or cytokinesis during meiosis. These irregularities may lead to morphological and genetic variation, which, in turn, may promote evolution or create intraspecific reproductive barriers (Kaur & Singhal 2019). Consequences may include atypical or male-sterile plants and impaired pollen formation, potentially affecting seed production standards and pollination success (Pozzobon et al. 2011).

Table 2. Post-meiotic products and meiotic index observed in six individuals of *Ochroma pyramidale*.

Individual	Tetrads	Monads	Dyads	Triads	Polyads	Meiotic index (%)
SP01	2,382	0	0	18	0	99.25 (0.84)*
SP02	2,381	2	6	10	1	99.21 (0.53)
SP03	2,360	7	16	17	0	98.33 (0.87)
IT04	2,358	15	11	16	0	98.21 (1.33)
IT05	2,386	0	0	14	0	99.42 (0.49)
AF06	2,206	0	15	178	1	91.92 (3.68)
Total	14,073	24	48	253	2	-
Mean	2,345.5	4	8	42.16	0.33	97.72
CV (%)				1.74		

CV: coefficient of variation. * In parentheses: deviations from the mean.

The dyes used to evaluate the pollen viability of *O. pyramidale* effectively distinguished viable from non-viable grains. With the Alexander's reagent, viable pollen exhibited intact violet-colored protoplasm and green cell wall contours (Figure 4A), whereas non-viable grains lacked protoplasm and appeared entirely green (Figure 4B). Using 2 % acetic carmine, viable pollen grains displayed red-stained protoplasm and intact cell walls (Figure 4C), whereas non-viable grains showed no staining (Figure 4D).

The summary of the analysis of variance for pollen viability, obtained via colorimetric testing, is shown in Table 3. A statistically significant difference was observed at the 1 % level between the two dyes, and at the 5 % level among individuals. However, no significant interaction was detected between the dye types and individuals.

The analyses performed with acetic carmine revealed that 96.16 % of the evaluated pollen grains were viable. Similarly, the colorimetric test using the Alexander's stain indicated a viability of 97.72 %. Although the individual IT04 presented the lowest mean viability, all individuals had values above 92.71 %, indicating a high percentage of viable pollen (Table 4).

Deviations from the mean ranged from 0.33 to 5.54 for acetic carmine and from 0.70 to 3.20 for the Alexander's stain (Table 4). The greatest deviations for viability using acetic carmine were observed in the individuals SP02, SP03 and IT04, whereas, for the Alexander's stain, the highest deviation was found in IT04. The Alexander's stain revealed a higher pollen viability percentage and a lower coefficient of variation, when compared to acetic carmine, indicating a greater experimental accuracy.

All individuals of *O. pyramidale* exhibited high pollen viability, regardless of the dye used. These findings are consistent with results reported for other tree species. Oliveira & Pierre (2018) observed pollen viability above 95 % in *Eugenia involucrata* using both acetic carmine and the Alexander's stain.

Table 3. Analysis of variance for the pollen viability of *Ochroma pyramidale* by colorimetric tests.

Source of variation	DF	MS
Dyes	1	58.1*
Individuals	5	390.7**
Dyes x individuals	5	67.4^{ns}
Mean	96.95 %	

DF: degree of freedom; MS: mean square for the variable pollen viability. ^{ns}, *, **: not significant, and significant at 5 % and 1%, respectively.

Table 4. Percentage of viable pollen grains of *Ochroma pyramidale* subjected to staining with acetic carmine and the Alexander's stain.

Individual	Pollen viability (%)	
	2 % acetic carmine	Alexander's stain
SP01	98.54 (0.90)	98.83 (1.08)*
SP02	94.92 (4.43)	98.38 (0.70)
SP03	94.04 (3.10)	97.92 (0.83)
IT04	92.71 (5.54)	93.54 (3.20)
IT05	97.33 (0.83)	99.04 (0.54)
AF06	99.42 (0.33)	98.58 (0.41)
Mean (%)	96.16	97.72
CV (%)	4.78	2.7

CV: coefficient of variation. * In parentheses: deviations from the mean.

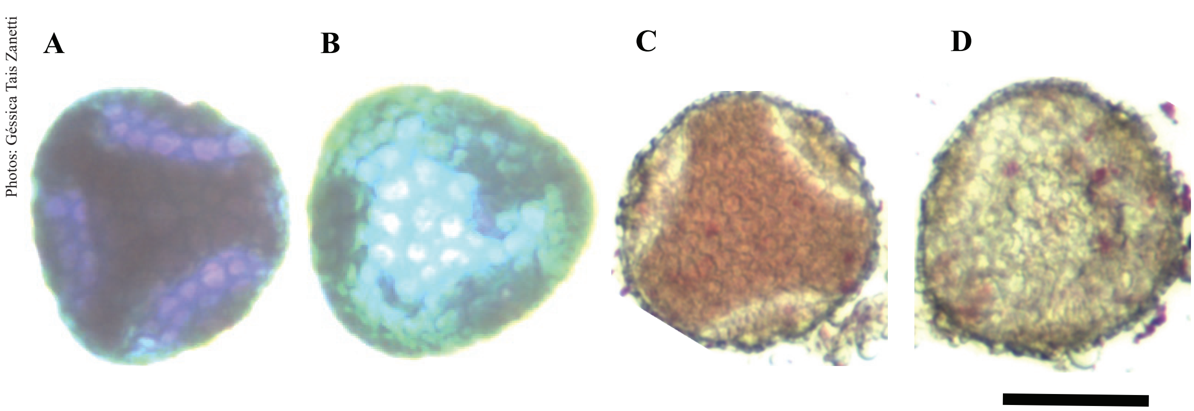

Figure 4. Pollen of *Ochroma pyramidale*. A) viable pollen stained with the Alexander's reagent; B) non-viable pollen stained with the Alexander's reagent; C) viable pollen stained with 2 % acetic carmine; D) non-viable pollen stained with 2 % acetic carmine. Bar = 100 µm.

Similarly, Santos et al. (2015) reported viability above 95 % in *Bertholletia excelsa* using the same dyes.

The present results also corroborate Zambrano et al. (2019), who evaluated *O. pyramidale* using the *in vitro* germination technique and confirmed a high viability rate, with no prevalence of sterile or non-viable pollen grains in any of the individuals. The higher viability values obtained using the Alexander's stain align with findings in other studies (Santos et al. 2015, Arenas-de-Souza et al. 2016, Hister & Tedesco 2016, Braga et al. 2018, Santos et al. 2019). The Alexander's stain facilitates the distinction between viable and non-viable grains, as aborted grains lack a nucleus and only the cellulose wall stains (Munhoz et al. 2008). Therefore, the use of the Alexander's stain is recommended for routine pollen viability assessments in *O. pyramidale*, as it is both effective and visually straightforward.

The high pollen viability found in *O. pyramidale* individuals is consistent with the high meiotic index observed, suggesting meiotic stability and regularity. This indicates a normal reproductive pattern and supports the findings of Lima et al. (2016), who stated that regular meiosis results in a high percentage of normal tetrads and, consequently, high pollen viability.

Lugol and Sudan IV dyes were effective in detecting starch and lipid, respectively, as reserve substances in the pollen grains of *O. pyramidale*. On average, 98.63 % of the pollen grains stained with Lugol showed a brown/yellow coloration, indicating the presence of starch (Figure 5A), whereas 98.57 % stained with Sudan IV showed a reddish coloration, indicating the presence of lipids (Figure 5B).

The presence of reserve substances such as starch and lipids in *O. pyramidale* pollen grains may have ecological and reproductive significance. Pacini et al. (2006) reported that starch, when converted into sugars and pectins, helps to maintain pollen viability by increasing resistance to adverse conditions and facilitating pollen tube germination, what may explain the high viability observed in this study. Lipids promote a better adhesion of grains to anthers and stigmas and serve as protection against dehydration and UV radiation (Pacini & Hesse 2005), which can also contribute to pollen viability.

The presence of starch and lipids in *O. pyramidale* pollen grains may also be associated with its pollination biology. Several authors suggest that reserve substances are related to ecological strategies and pollinator interactions (Dettke & Santos 2011, Oliveira & Pierre 2018). Reis & Paludzyszyn Filho (2011) reported that *O. pyramidale* is primarily pollinated by nectar-seeking bats. Lipids may provide a nutritional reward for pollinators, as noted by Pacini & Hesse (2005), and bat visits may also involve pollen consumption, a nutrient-rich resource containing amino acids, polysaccharides, lipids, vitamins and proteins (Mancina & Gerardo-Herrera 2010, Teixeira et al. 2014).

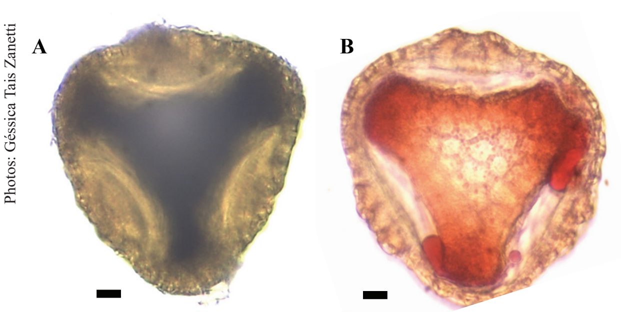

Figure 5. Pollen grains of *Ochroma pyramidale* subjected to different staining methods for chemical composition analysis. A) Starch-positive pollen grain (brown/yellow coloration); B) lipid-positive pollen grain (reddish coloration). Bar = 20 µm.

CONCLUSIONS

1. Meiotic regularity and viable gamete production in *Ochroma pyramidale* are directly associated with reproductive success and species maintenance;
2. All individuals evaluated through cytological analysis exhibited gametic stability and can be recommended for seed production.

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