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Original Article

Reproductive biology of *Talisia esculenta* (Cambess.) Radlk. (Sapindaceae)

Biologia reprodutiva de *Talisia esculenta* (Cambess.) Radlk. (Sapindaceae)

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Abstract

The family Sapindaceae comprises species with different reproductive strategies and sexual systems; however, dioecy is rarer and has been little investigated in tree species. We analyzed the reproductive biology of *Talisia esculenta* (Cambess.) Radlk. to understand its sexual system and reproductive potential. For this purpose, we examined floral biology and the reproductive system in natural populations of *T. esculenta*. The species exhibits two sexual morphs: individuals with morphologically hermaphroditic but functionally pistillate flowers, characterized as female, and individuals with staminate flowers, characterized as male. The hermaphroditic flowers produce viable pollen but do not self-pollinate due to strong hercogamy and the permanent indehiscence of the anthers; therefore, they are functionally female. These characteristics classify *T. esculenta* as an allogamous and androdioecious species, specifically a functionally dioecious type. Natural and hand pollination between male and female individuals resulted in a higher percentage of fruit set with significantly superior quality compared to fruits from hand cross-pollination between female individuals. However, the fruit set rate in these crosses was only 25% of the floral investment. The species is an obligatory xenogamous and invests in a high floral display (75% of the flowers) to attract pollinators. This is a secondary sexual trait that favors pollination. Pollination tests indicate that local pollinators are effective, suggesting that other factors may influence the low natural fruit set rate. Genetic and phylogenetic studies are necessary to further understand androdioecy and the reproductive behavior of *T. esculenta*.

Keywords: androdioecy, dioecy, hermaphrodite flowers, staminate flowers, pitomba.

Resumo

A família Sapindaceae possui espécies com diferentes estratégias reprodutivas e sistemas sexuais, no entanto, a dioicia é mais rara e tem sido pouco investigada em espécies arbóreas. Analisamos a biologia reprodutiva de *Talisia esculenta*, com o objetivo de compreender o seu sistema sexual e potencial reprodutivo. Para isso, analisamos a biologia floral e o sistema reprodutivo em populações naturais de *T. esculenta*. A espécie tem dois morfos sexuais: indivíduos com flores morfologicamente hermafroditas, mas funcionalmente pistiladas, caracterizados como femininos e indivíduos com flores estaminadas, caracterizados como masculinos. As flores hermafroditas produzem pólen viável, mas não se autopolinizam devido à forte hercogamia e indeiscência permanente das anteras, portanto, são funcionalmente femininas. Essas características classificam *T. esculenta* como uma espécie alógama e androdioica, do tipo dioica funcional. A polinização natural e a manual entre indivíduos masculinos e femininos formou maior porcentagem de frutos com qualidade significativamente superior do que os frutos da polinização cruzada manual entre indivíduos femininos. Mesmo assim, a taxa de formação de frutos nesses cruzamentos foi de apenas 25% do investimento floral. A espécie é xenogâmica obrigatória e investe no elevado display floral (75% das flores) para atração de polinizadores. Essa é uma característica sexual secundária que favorece a polinização. Os testes de polinização indicam que os polinizadores locais são efetivos, sugerindo que outros fatores podem influenciar na baixa taxa de frutificação natural. Estudos genéticos e filogenéticos são necessários para compreender a androdioicia e o comportamento reprodutivo de *T. esculenta*.

Palavras-chave: androdioicia, dioicia, flores hermafroditas, flores pistiladas, pitomba.

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1. Introduction

Dioecy is a sexual system characterized by the production of exclusively staminate or pistillate flowers in separate individuals of the same species (Tanurdzic and Banks, 2004; Lenza and Oliveira, 2005). The coexistence of individuals producing staminate and hermaphroditic flowers in the same population characterizes androdioecy, an uncommon reproductive system in nature (Pannell, 2002), which most likely evolved from dioecy (Delph and Wolf, 2005; Delph, 2009) rather than hermaphroditism (Wolf and Takebayashi, 2004).

The *Sapindaceae* family is mostly composed of monoecious species, and less commonly dioecious or polygamous ones (Avalos et al., 2017; Solís et al., 2017). Functionally bisexual flowers are rare in species of this family (Acevedo-Rodríguez et al., 2010); however, staminate flowers with rudimentary gynoecium and hermaphroditic flowers with non-functional stamens are common characteristics of monoecious and dioecious species (Ferrucci and Somner, 2005).

Species of *Sapindaceae* such as *Magonia pubescens* A. St.-Hil. and *Allophylus edulis* (A. St.-Hil.) Hieron. ex Niederl. have staminate flowers with pistillode gynoecium and degenerate ovules in the early stages of their development. In contrast, in morphologically hermaphrodite but functional pistillate flowers, the anthers are indehiscent at maturity (González at al., 2014; González at al., 2017). Functional pistillate flowers of *Acer elegantulum* Fang et PL Chiu (Sapindaceae) have stamens with shorter filaments and larger anthers that are always indehiscent (Luo et al., 2017), while *in Paullinia weinmanniifolia* Mart. (Lima et al., 2016) and *Koelreuteria elegans* subsp. formosana (Hayata) F.G. Mey. (Avalos et al., 2017), in addition to indehiscent anthers, pollen grains are inviable.

Hermaphroditic flowers of *Tina striata* Radlk. (Sapindaceae) produce pollen grains with the same viability, pollen structure, number, and type of openings as staminate flowers. However, the anthers do not become dehiscent and thus do not release pollen grains. Moreover, the pollen from hermaphroditic flowers of *T. striata* has a lower capacity to adhere to and develop pollen tubes on the stigma than the pollen grains from staminate flowers (Vary et al., 2011). In *Acer oblongum* Wall. ex DC. (Sapindaceae), the pollen of morphologically hermaphroditic flowers has significantly reduced viability and insufficient potential to fertilize ovules, in addition to being unable to germinate on the stigma of the same flower (Yadav et al., 2016). The viability of pollen grains used in pollination directly influences fertilization success, as the relationship between pollen viability, germination capacity, and fruit formation is positively linear (Sulusoglu and Cavusoglu, 2014). This is a point that should be considered in the study of *Sapindaceae* species.

Although the *Sapindaceae* family is highly diverse (Zini et al., 2012) and widely distributed in tropical and subtropical regions (Acevedo-Rodríguez et al., 2017), the research on reproductive biology and pollination systems has focused on a limited number of species. Most of the available studies in the literature include Asian species of the genus *Acer*, cosmopolitan species of herbs or climbers.

The species *Talisia esculenta* (Cambess.) Radlk. (Sapindaceae) is a fruit tree native to the Amazon, but also widely distributed in the Caatinga, Cerrado, and Atlantic Forest of Brazil (Acevedo-Rodríguez, 2015). It has high potential for use in construction and commercial forestry, as well as for reforestation in riparian forest areas (Lorenzi, 2016). Despite its economic importance, there is little information on the reproductive biology of this native species. Therefore, we studied the reproductive biology of *T. esculenta* in natural populations to identify its sexual system and reproductive potential, generating information that will aid in the management of this species.

2. Material and Methods

2.1. Study area and sampling

We selected thirty-two adult individuals of *T. esculenta* (twelve males and ten females) in natural populations in the municipality of Areia (6°58'12"S and 35°42'15"W), Paraíba, Brazil. The region has an annual average temperature of 22 °C, relative humidity of 85%, and annual precipitation of 1400 mm (AESA, 2018).

We monitored *T. esculenta* individuals in the flowering stage, with good phytosanitary and physiological conditions during the periods of January to February 2017 and December to February 2019. A voucher of *T. esculenta* is deposited in the Jayme Coêlho de Moraes Herbarium (EAN) identified as 'BRASIL. Paraíba: Areia, s.d., M.L.M. Silva s.n.' (EAN 25774) as a voucher specimen.

2.2. Floral biology

We collected inflorescences (n = 100) carrying pre-anthesis floral buds and anthesis flowers from five male and five female individuals, which were fixed in 70% ethanol for subsequent analysis of floral biology in the Laboratório de Ecologia e Reprodução Vegetal at the Centro de Ciências Agrárias, Universidade Federal da Paraíba.

We measured the mean number of flowers per inflorescence by sex (n = 10 per individual), inflorescence length, corolla length, corolla diameter, and carpel length using a digital caliper (accuracy ±0.01 mm). The length of stamens in flowers of male and female individuals was determined using a stereomicroscope with a micrometer ocular. Three inflorescences per individual (n = 5 individuals of each sex) with a total of 10 flowers per inflorescence were used to measure these parameters.

We determined the anthesis period by marking 10 pre-anthesis floral buds from different individuals and sexes and continuously monitoring them from 6:00 am to the end of the anthesis. We determined the presence of nectar in male and female flowers of T. esculenta through visual confirmation using a printed color scale from the Accu-Chek Active glucose test strip container. To achieve this, we bagged floral buds (n = 20 for each sex) from different individuals the day before anthesis. Ten buds were used to verify nectar presence at 6:00 am and ten remained bagged for assessment at the end of anthesis. We tested stigma receptivity by applying hydrogen peroxide (H_2O_2) to the stigma of 10 flowers per female individual at the onset of

anthesis (Galen and Plowright, 1987; Dafni et al., 2005). The characteristic aroma emitted by male and female flowers of the species was documented using human olfactory perception. During field observations, we recorded the presence and absence of flowering and fruiting events in female and male individuals of the matrix plants.

We collected three inflorescences per female individual and evaluated ten floral buds/flowers in the pre-anthesis, anthesis, and post-anthesis stages, through a stereomicroscope, to verify the anther dehiscence of flowers from female individuals (morphologically hermaphroditic). We collected ten anthers from pre-anthesis flower buds from five male and five female individuals. We macerated the anthers on a microscope slide following the protocol of Guerra and Souza (2002), with a drop of Alexander's reagent (Alexander, 1980) added. After, a 1 mm^2 square cover slip was placed over the sample. The slides were examined under an optical microscope, and a hand counter was used to determine the number of viable, non-viable, and total pollen grains per flower, as well as pollen viability. Pollen grains that turned intensely pink were counted as viable (with cytoplasm) and those stained light blue as non-viable (without cytoplasm) (Kearns and Inouye, 1993). We determined the pollen-to-ovule ratio (P/O) according to Cruden (1977) using ten flowers per inflorescence/sex.

2.3. Reproductive system

We conducted controlled pollinations on flowers ($n \geq 10$) from three inflorescences per female individual, totalling 30 inflorescences for each treatment: (1) natural pollination (NP, control), (2) hand cross-pollination from male to female individuals (M × F), and (3) hand cross-pollination between female individuals (F × F).

In the NP treatment, we counted and marked pre-anthesis floral buds to monitor fruit development. In the hand cross-pollination treatments, we bagged female pre-anthesis buds to prevent contact with floral visitors. The recipient flowers were not emasculated, as the anthers of female individuals remain indehiscent throughout anthesis. After flower opening, we performed the crosses using brushes and then bagged the inflorescences. We monitored fruit formation for 30 and 60 days after pollination. We determined the fruit abortion rate at 60 days after anthesis.

We calculated reproductive efficacy (RE) as the ratio of the percentage of fruits set through natural pollination to those set through cross-pollination. RE values below 0.66 indicate low reproductive efficacy, whereas values above 0.81 indicate high reproductive efficacy (Zapata and Arroyo, 1978).

The fruit production potential of the species was assessed based on the following characteristics of fruits resulting from natural and cross-pollination tests: the number of fruits per inflorescence (recorded at 60 days post-pollination), fruit size (length, width, and thickness), and fruit weight.

2.4. Statistical analysis

A completely randomized experimental design was used. We compared the means of reproductive structures between male and female individuals using a t-test, following a normality assessment of the data. We conducted a comparison between natural pollination and hand cross-pollination data using analysis of variance (ANOVA), after verifying data normality. The regression model with the highest significance and best fit to the data was applied. When applicable, means were compared using Tukey's test. All analyses were performed in the SAS 9.2 statistical software (Statistical Analysis Systems, 2011).

3. Results

3.1. Floral biology

Terminal axillary inflorescences of *T. esculenta* exhibit significant morphometric differences between female and male individuals. Male individuals produce inflorescences approximately twice the size of those produced by females and with significantly more flowers (Table 1; Figures 1A-B). In the same inflorescence, we observed floral buds and flowers at different stages (pre-anthesis, anthesis, and floral senescence).

The female individuals produce inflorescences with morphologically hermaphrodite flowers that are white, sweet-scented, and easily detected by the human sense of smell. These flowers contain three ovules and eight stamens (Table 1) that are inserted in the receptacle above the floral nectary, adhered to the base of the gynoecium, characterizing a strong herkogamy in these flowers. The stamens have subsessile fillets and indehiscent anthers (Figure 1C) at all stages (pre-anthesis, anthesis, and floral senescence) and are present in all evaluated flowers.

The inflorescences of male individuals produce exclusively staminate flowers, which are also white and sweet-scented. The flowers exhibit a significantly smaller mean corolla diameter than hermaphroditic flowers and

Table 1. Morphometry of inflorescences and flowers of *Talisia esculenta*, Paraíba, Brazil. Mean values of inflorescence length (IL, cm), number of flowers per inflorescence (NF/I), corolla length (CL, cm), corolla diameter (CD, cm), carpel length (CaL, cm), stamen length (SL, cm), and number of ovules (O) and stamens (S) per flower.

Individuals	IL	NF/I	CL	CD	CaL	SL	O	S
Female	11.52	37.64	0.59	0.45	0.44	0.21	3	8
Male	21.43	170.68	0.64	0.36	-	0.47	-	8
t-test (p)	0.0083*	0.0004*	0.3858ns	0.0010*	-	0.0000*	-	-

*Significant at 5% probability ($p < 0.05$) by t-test. nsNot significant ($p > 0.05$).

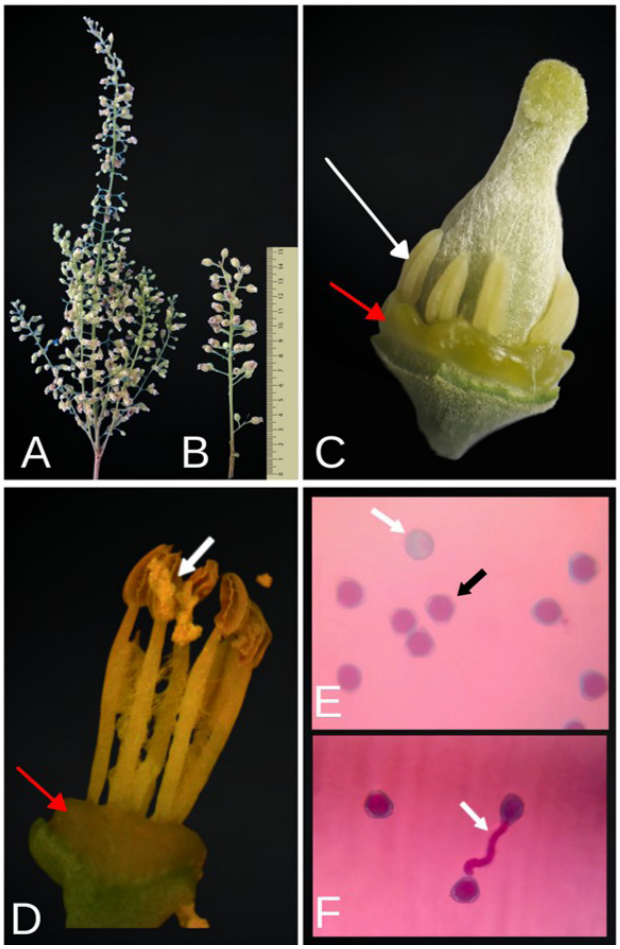

Figure 1. Floral biology of *Talisia esculenta* (Cambess) Radlk. - A: Male inflorescence. B: Female inflorescence. C: Carpel of the functionally female flower (short stamens with indehiscent anthers surrounding the carpel - white arrow). D: Male flower (with long, pubescent stamens and dehiscent anthers - white arrow). E: pollen grains (viable - black arrow and non-viable - white arrow). F: Germination of the pollen tube from male flowers (pollen tube - white arrow). Red arrows indicate the nectary disk of flowers in C and D.

feature eight stamens inserted into the receptacle, which are significantly larger than those found in flowers of female individuals (Table 1). The stamens have long, pubescent fillets and anthers with longitudinal dehiscence during the anthesis period (Figure 1D). Additional information regarding floral morphometry is detailed in Table 1.

We did not recorded synchrony in flowering and fruiting events among female individuals. This was evidenced by the presence, within the same population and during the same period, of female individuals bearing mature fruits, developing fruits, and others in flowering. On the other hand, we recorded male and female individuals flowering simultaneously within the same population.

The anthesis of male and hermaphroditic flowers of *T. esculenta* begins at sunrise, approximately at 05:00 am, and lasts for about 30 hours. Floral senescence is characterized by petal darkening and wilting, with the complete exposure of the carpel in hermaphroditic flowers. The opening of staminate and hermaphroditic flowers occurs as the petals unfurl from the inside out; however, they do not fully open. Consequently, in morphologically hermaphroditic flowers, the anthers remain covered by the petals, as the stamens are shorter than half the length of corolla. Moreover, the anthers are indehiscent during anthesis, classifying these flowers as functionally pistillate. In staminate flowers, the anthers are exposed, and during anthesis, a yellowish, adhesive mass of pollen grains is released (Figure 1D). The presence of nectar in staminate and hermaphroditic flowers was recorded during anthesis in all evaluated flowers. The highest stigma receptivity index occurred between 09:00 am and 03:00 pm, characterized by the formation of small bubbles in approximately only 30% of the flowers evaluated.

The pollen grains of staminate flowers have three openings (tricolpate pollen grains), which was impossible to identify in the pollen grains of functional pistillate flowers, which have a more rounded shape. The total, viable, and non-viable pollen grains of *T. esculenta* (Figure 1E) were higher in staminate flowers, with a significant difference observed only in the higher number of non-viable pollen grains (Table 2). Although the P/O ratio did not differ significantly between floral morphs, pollen viability was significantly higher in functionally pistillate flowers (Table 2).

3.2. Reproductive system

The pollination type plays a crucial role in determining the quantity and quality of fruits produced by *T. esculenta* (Table 3). Natural pollination (control) and hand cross-pollination between male and female individuals (M x F) resulted in a higher number of fruits formed, with greater average of weight, length, width, and thickness. The fruits obtained from hand cross-pollination among female individuals (F x F) were significantly smaller in size and weight (Table 4). Thirty days after pollination, fruit formation was observed in all treatments. However, sixty days after pollination, an abortion rate of 4.6%, 3.9% and 0.61% were recorded in the control and hand cross-pollination (M × F), (F x F) treatments, respectively. The reproductive efficacy index (RE) was high in both pollination systems, with values of 1.06 for hand cross-pollination (M × F) and 3.03 for hand cross-pollination (F × F). However, the fruit set at 60 days did not exceed 25% of the pollinated flowers.

4. Discussion

The inflorescences of male individuals of *T. esculenta* are visibly larger, more branched, with a more abundant flower production than morphologically hermaphroditic flowers, which makes them more attractive to pollinators. The greater abundance of exclusively staminate flowers in the species increases attractiveness to pollinators, ensuring pollination within populations (gene flow) (Yadav et al., 2016). In dioecious species, male individuals typically exhibit a greater floral display than females, characterized by a higher flower abundance, larger floral structures, and greater visual conspicuousness (Barrett and Hough, 2013).

The lengths of inflorescence and stamens were greater in the staminate flowers. However, the corolla diameter was significantly larger in the functionally pistillate flowers, probably due to the presence of the carpel with an oval-shaped ovary surrounded by stamens. The differences in size and attractiveness between staminate and pistillate flowers are secondary sexual traits that function as adaptive strategies, allowing for directional pollen flow to conspecific stigmas (Grant, 1995; Barrett and Hough, 2013). In addition,

Table 2. Floral biology of *Talisia esculenta*, Paraíba, Brazil. Mean values (per flower) of the total pollen grain number (TP), viable pollen (VP), and non-viable pollen (NVP), percentage of pollen viability (PV), and pollen/ovule ratio (P/O) in natural populations of *Talisia esculenta* in Paraíba, Brazil.

Individuals	TP	VP	NVP	PV (%)	P/O
Female	19648.91	19587.31	61.60	99.94	6549.77
Male	22146.77	21855.84	290.93	99.84	7382.26
t-test (p)	0.08443^{ns}	0.10137^{ns}	0.00771*	0.04543*	0.08451^{ns}

^{ns}Not significant ($p > 0.05$). *Significant at 5% probability ($p < 0.05$) by t-test.

Table 3. Analysis of variance for fruit set at 30 and 60 days after pollination (F30d and F60d, respectively) and fruit weight (FW), length (FL), width (FWd), and thickness (FT) from hand cross-pollination and natural (control) pollination systems of *Talisia esculenta* in Paraíba, Brazil.

Source	GL	Mean square					
		F30d	F60d	FW	FL	FWd	FT
Pollination	2	0.8349*	0.6175*	1.4232*	3.1550*	3.5112*	3.1432*
Residual	87	0.06325	0.06075	0.12549	0.24109	0.27089	0.23784
Mean		0.42	0.38	0.75	1.06	1.3	1.06
CV (%)		59.93	65.07	47.05	46.16	46.16	46.11

*Significant at 5% probability ($p < 0.05$) by Tukey-test.

Table 4. Pollination treatments. Number of pollinated flowers (PF), fruiting at 30 and 60 days after pollination (F30d and F60d, %), and average fruit weight (FW, g), length (FL, cm), width (FWd, cm), and thickness (FT, cm) from handly cross-pollinated and naturally pollinated (control) *Talisia esculenta* in Paraíba, Brazil.

Treatments	PF	F30d	F60d	FW	FL	FWd	FT
Natural pollination (control)	1319	28.49 ^{a}	23.89 ^{a}	7.85 ^{a}	2.15 ^{a}	2.61 ^{a}	2.11 ^{a}
Hand cross-pollination (M x F)	628	26.79 ^{a}	22.89 ^{a}	6.70 ^{a}	1.78 ^{a}	2.17 ^{a}	1.76 ^{a}
Hand cross-pollination (F x F)	403	9.38 ^{b}	8.77 ^{b}	4.47 ^{b}	1.11 ^{b}	1.34 ^{b}	1.07 ^{b}

Identical letters do not differ at a 5% probability level according to Tukey's test. M = male; F = female. Mean test: a = treatments of greatest significance; b = treatments of least significance.

the arrangement of flowers in inflorescence can increase the attraction of pollinators through the increase in floral display and can also function as a landing area for floral visitors (Proctor et al., 1996).

The lower pollen viability and the higher proportion of non-viable pollen grains in staminate flowers are offset by producing a greater number of flowers per inflorescence, which ensures successful pollination (Rosado et al., 2018). Moreover, although the viability is higher in functional pistillate flowers, their stamens have indehiscent anthers, and therefore the pollen grains are not available for pollination. The high pollen/ovule ratio observed classifies the species as obligate xenogamous (Cruden, 1977), indicating that it requires a biotic vector for pollination. The low proportion of stigma receptivity observed in the species coincided with the number of fruits formed 60 days after pollination, suggesting that the plant allocates 70% of its female flowers to improve floral display and increase visits of the pollinators.

The main difference between floral morphs of the *T. esculenta* lies in the stamens, which are shorter with indehiscent anthers in functionally female individuals and more exposed with dehiscent anthers in male individuals. This difference enhances the likelihood of pollen transfer from male to functionally female individuals, thereby reducing the chances of cross-pollination among female individuals, where the fruit set rate is significantly lower (Table 4). The smaller androecium in hermaphrodite flowers than in staminate flowers suggests a loss of male function in these flowers (Vary et al., 2011). This confirms the requirement for a biotic vector for effective pollination and fruit formation, as self-pollination is not possible due to strong herkogamy and indehiscence of anthers in morphologically hermaphrodite flowers. Based on the analysis of floral attributes and resources, *T. esculenta* can be classified as a melittophilous species (Faegri and Pijl, 1979), characterized by flowers with a strong, sweet fragrance, the presence of nectar, and anthers with attractive yellow pollen mass (in staminate flowers) (Culley et al., 2002).

Other species of the Sapindaceae family, such as *Acer elegantulum* Fang et PL Chiu, also have the same morphological pattern observed in *T. esculenta*, i.e., hermaphrodite flowers with significantly shorter filaments and larger always indehiscent anthers (Luo et al., 2017). The production of morphologically hermaphrodite flowers with always indehiscent anthers, but with similar number of pollen grains and viability as staminate flowers, observed in *Talisia esculenta*, has also been reported in other *Sapindaceae* species, such as *Tina striata* Radlk., in which the indehiscent anthers of female flowers produced viable pollen grains, but with reduced germination capacity and low potential to fertilize ovules (Vary et al., 2011). In the case of *Paullinia weinmanniifolia* Mart., the production of morphologically bisexual and functionally unisexual flowers is present; however, the pollen produced in the indehiscent anthers of hermaphrodite flowers is 100% inviable (Lima et al., 2016).

The floral morphs characterize *T. esculenta* as an androdioecious species of the functional dioecy type (Lloyd, 1979), given that, despite having hermaphroditic flowers with viable pollen, they are not available for pollination due to the permanent indehiscence of the anthers. Androdioecy is considered a rare sexual system in Angiosperms (Lloyd, 1975; Charlesworth, 1984), present in a few woody species that evolved from hermaphroditic ancestors and in herbaceous species with dioecious ancestors (Lopes et al., 2018). However, detailed studies of the reproductive system and phylogenetic studies are necessary to determine if androdioecy in *T. esculenta* evolved from dioecy or hermaphroditic ancestors.

In most of the species of Sapindaceae mentioned in the literature, the sterilization of one sex begins late in meiotic development, resulting in male unisexual flowers with sterile carpels (carpeloids) and female flowers with sterile stamens (staminoids) (Cao et al., 2017). By analyzing anthers of staminate and pistillate flowers of *Melicoccus lepidopetalus* Radlk., was observed that young anthers are similar in both floral types but differ anatomically when mature (Zini et al., 2012). According to these authors, in mature anther of staminate flowers, there are compressed and endothecial cells with fibrillar thickenings, while in mature anthers of functional pistillate flowers, the epidermis is not compressed, the endothecium does not develop fibrillar thickenings, the middle layers and the tapetum generally persist, and the stomium is not functional, which prevents anther dehiscence. Similar results were observed for *Magonia pubescens* (González et al., 2017), in which staminate and hermaphroditic flowers are morphologically similar but anatomically different.

In some species, retaining androecium in functionally pistillate flowers may confer an adaptive advantage by increasing their attractiveness to pollinators (Bawa, 1977). However, this does not appear to be the case for *T. esculenta*, as the stamens of its hermaphroditic flowers are fully protected by the sterile floral whorls, not being seen by pollinators during anthesis. The presence of hermaphroditic flowers that are functionally pistillate in female *T. esculenta* individuals suggests that there has not been sufficient evolutionary time for the removal of stamens in these flowers. Consequently, this floral morphology is considered a transitional state in the evolution from unisexuality to dioecy (Mayer and Charlesworth, 1991).

The low fruit set in *T. esculenta* may be due to yet unknown genetic-level reproductive strategies of the species. These results highlight the need for studies to identify and assess possible incompatibility mechanisms in *T. esculenta*. Clearly, the species invests heavily in flower production to attract pollinators (75% of flowers), but its reproductive success rate averages only 25%, which requires further investigation. In *Cupania vernalis* Camb. (Sapindaceae), Ferreira (2009) also observed a considerable number of flower abscissions and abortions in natural pollination and self-pollination tests, attributed to ineffective pollination. However, in the case of *T. esculenta*, hand pollination tests ensure effective pollination of flowers, and the lack of a significant difference between natural and hand cross-pollination (M × F) indicates that local pollinators are efficient.

In the hand cross-pollination experiment between different female individuals (F × F), the abortion rate was lower, and reproductive efficacy was higher compared

to the other treatments. However, the fruits were of low quality, and the fruit set rate was significantly lower. Additionally, fruit formation does not naturally occur due to strong herkogamy and the permanent indehiscence of anthers in the flowers of female individuals. Therefore, *T. esculenta* is an obligate xenogamous species, allogamous and is dependent on pollinators.

5. Conclusion

The flowers of male *T. esculenta* plants are exclusively staminate, while those of female plants are morphologically hermaphroditic but functionally pistillate. The main difference between these morphs is the presence of long stamens with dehiscent anthers in staminate flowers, whereas in functionally pistillate flowers, there is strong herkogamy and the stamens have indehiscent anthers. Therefore, *T. esculenta* is classified as an androdioecious species of the functional dioecious type. The species is melittophilous, an obligate xenogamous, allogamous and is dependent on pollinators. *T. esculenta* exhibits a low natural fruit set, highlighting the need to investigate potential reproductive strategies at the genetic level. Hand pollination treatments suggest that local pollinators are effective, indicating that other factors may be influencing natural fruit production.

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