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BIOLOGY

Morphometry of fruits and pyrenes in two morphotypes and populations of *Butia purpurascens* Glassman (Arecaceae)

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ABSTRACT: *Morphometry of fruits and seeds is a useful and important information for phenotyping and genetic characterization of crop plant species and, for conservation of endangered species, as is the case of Butia purpurascens Glassman. In this study, we determined comparatively the morphometric features of fruits and pyrenes between yellow and magenta B. purpurascens morphotypes, located in four different sites (populations). We processed 1380 fruits collected from 69 individuals. We evaluated the length, width and weight of the fruits and pyrenes, the fresh and dry pulp mass, and percentage of pulp moisture. Yellow morph showed larger and heavier fruits than magenta morph. Morphometric differences were also evident among the four populations, suggesting that ecosystems fragmentation can cause deleterious genetic effects in B. purpurascens in long-term.*
Key words: *Cerrado, habitat fragmentation, morphometric variation, palm tree, reproductive isolation.*

Morfometria de frutos e pirênios em dois morfotipos e populações de *Butia purpurascens* Glassman (Arecaceae)

RESUMO: *A morfometria de frutos e sementes é uma informação útil e importante para fenotipagem e caracterização genética de espécies vegetais com potencial econômico e, para a conservação de espécies ameaçadas, como é o caso de Butia purpurascens. Nesse estudo, determinamos comparativamente as características morfométricas de frutos e pirênios entre morfotipos amarelo e magenta de B. purpurascens, localizados em quatro localidades diferentes (populações). Beneficiamos 1380 frutos, coletados em 69 indivíduos. Avaliamos o comprimento, largura e o peso dos frutos e pirênios, a massa fresca e seca da polpa, e a porcentagem de umidade da polpa. O morfotipo amarelo apresentou frutos maiores e mais pesados do que o morfotipo magenta. Diferenças morfométricas também foram notórias entre as quatro populações, sugerindo que a fragmentação de ecossistemas pode causar efeitos genéticos deletérios em B. purpurascens ao longo do tempo.*
Palavras-chave: *Cerrado, fragmentação de habitats, variação morfométrica, palmeira arborescente, isolamento reprodutivo.*

INTRODUCTION

The morphometric characterization of fruits and seeds is important in taxonomic studies, identification of varieties with economic value, verification of the phenotypic and genetic variation occurrence and association with environment, both within and between plant populations (SILVA et al., 2007; FELIZARDO et al., 2015; PADILHA et al., 2016; RIOS et al., 2016). Also provides support for research on fruit production and plant propagation (SCHLINDWEIN et al., 2019), since the degradation of natural ecosystems reduces the quality of progenies on a global scale (AGUILAR et al., 2019), and in the Brazilian Cerrado (MATOS et al., 2014; PALERMO & SOUZA, 2019).

The Cerrado is recognized as the most diverse savannah in the world and a worldwide *hotspot* (KLINK & MACHADO, 2005). Over the years, agricultural pressure has led to habitat loss, resulting in the fragmentation of ecosystems. This is one of the most important threats to the maintenance of natural resources and biodiversity (GUILHERME et al., 2020). Palm species contribute significantly to this diversity, and are important in the characterization and structuring of several physiognomies not only in the Cerrado, but also in the Neotropics; although, there are still gaps in the knowledge about them (MUSCARELLA et al., 2020).

The Arecaceae family comprises about 252 genera and 2600 species distributed in the

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tropics and subtropics (DRANSFIELD et al., 2008), with 71 genera and 376 species registered for Brazil (LEITMAN et al., 2015). *Butia* encompasses 19 South American species, four of them typical of open physiognomies of the Brazilian Central Plateau (HEIDEN et al., 2020). One of them is *B. purpurascens*, which have individuals with varying colors in the inflorescence axis, floral structures and fruits (GUILHERME et al., 2015), ranging from yellow to magenta (Figure 1). It is important to know the extent to which these variations, treated as morphotypes, differ morphologically and limit the species sexual reproduction. According to WENDT et al. (2011) colors variations in reproductive structures of *Euterpe*, a genus of the Arecaceae, may indicate different phenological patterns and future reproductive isolation between morphs.

Although the fruits and seeds morphometry in palm is well studied (SILVA et al., 2007; MANFIO et al., 2011; MATOS et al., 2014; SANTOS et al., 2016), including *Butia* species, such as *B. capitata* (PEDRON et al., 2004; MOURA et al., 2010; SILVA & SCARIOT, 2013), there are no studies on *B. purpurascens*. This species occurs mainly in the southwest of the Goiás State, Brazil (GUILHERME & OLIVEIRA, 2011), and shows economic potential, with extraction of leaves and fruits for making brooms and consumption, respectively (GUILHERME et al., 2015), besides its ornamental application. A recent study showed high seed predation by curculionids, low germination rates and slow plant development under controlled conditions (RESSEL & GUILHERME, 2022). Based on that, *B. purpurascens* has been reclassified as critically endangered (CNCFLORA, 2020) and; therefore, further studies are needed for the species conservation.

In this sense, the morphometric evaluation can be important for selection of varieties within the species, which allows its cultivation and consequent sustainable commercial exploitation (PASSOS et al., 2014). In addition, morphometric differences in plant reproductive structures may indicate population isolation, caused by the habitat fragmentation in Cerrado, as found for example in *Mauritia flexuosa* L.f. (MATOS et al., 2014) and *Annona crassiflora* Mart. (PALERMO & SOUZA, 2019). Habitat loss and fragmentation can reduce gene flow and genetic diversity in plants, disrupting seed dispersal (BROWNE & KARUBIAN, 2017). Additionally, studies on the effects of fragmentation of natural ecosystems have shown that extractive practices, defaunation and hunting can severely limit the establishment and maintenance of palm species (GALETTI et al., 2006; FLEURY & GALETTI, 2006).

The present study had the follow objectives: (i) evaluate comparatively the fruit

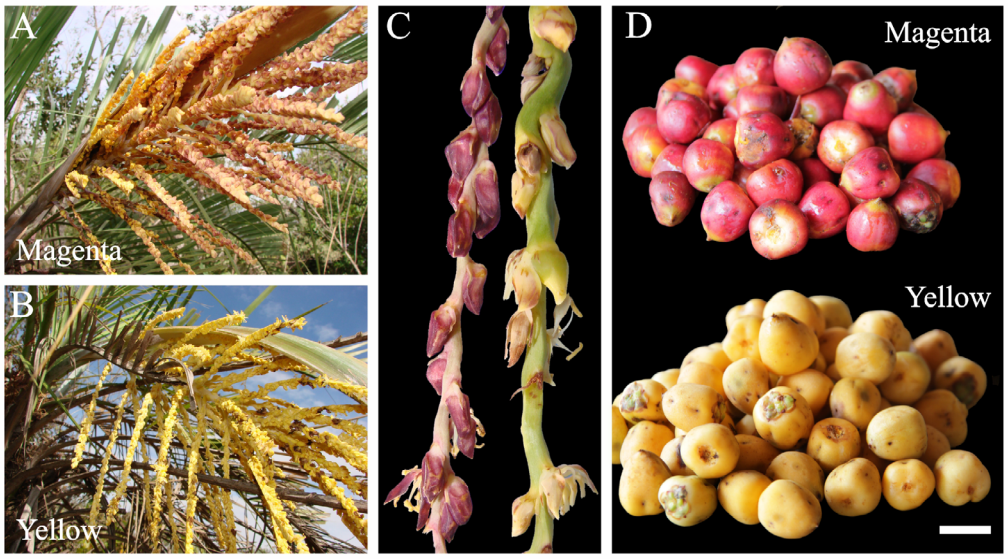

Figure 1 - Coloring patterns of magenta (A) and yellow (B) morphs inflorescences, floral rachis (C) and fruits (D) of *Butia purpurascens*. Bar = 20 mm.

and pyrenes morphometric features of yellow and magenta *B. purpurascens* morphotypes. (ii) evaluate comparatively the morphometric features of fruit and pyrenes of four isolated *B. purpurascens* populations in order to identify and discuss possible effects of habitat loss and fragmentation.

MATERIALS AND METHODS

Species characteristics

Butia purpurascens Glassman is a palm tree endemic of Brazil with distribution center in the southwest Goiás, and surrounding localities of Mato Grosso do Sul and Minas Gerais states (RESSEL & GUILHERME, 2022), and locally known as 'butiá', 'palmeira-jataí' or 'coqueiro-de-vassoura'. Adult plants have strongly arched leaves, single and exposed stipe, usually with stature of 3-4 m; although some individuals can reach 7 m (HEIDEN et al., 2020). The species is monoecious, with inflorescences showing male and female flowers. The drupe fruits are ovoid with a succulent and aromatic mesocarp, and the hard endocarp with one seed had three germinative pores. The fructification period occurs from October to December (GUILHERME et al., 2015).

Study site and populations

We performed the fruits gathering for morphometric variables in four sites (populations) in the municipality of Jataí, southwest Goiás (Table 1). The populations (P1, P2, P3 e P4) are reported in private properties, previously occupied by cerrado *stricto sensu*, which have become cattle pastures, remaining isolated native trees and several individuals of *B. purpurascens*. Therefore, the four areas undergo strong anthropic pressure and are in a matrix dominated by monocultures, especially soybeans and corn, one of which also surrounds the urban perimeter. Thus, is likely the loss of the seed dispersers.

The largest distance among the four populations is 22 km; and therefore, have the same climatic condition. The climate of Jataí is classified as Aw, according to Köppen (ALVARES et al., 2013), with seasonality marked by drought in winter and rain in summer. The annual average rainfall is approximately 1600-1700mm, with rainfall occurring mainly between the months of October and April. In the rainy season, the moisture is always greater than 70%, with higher average temperatures. In the dry period, the average temperature approaches 18 ºC, in which the relative moisture does not exceed 50%, with extremes occurring in June and July. Oxisol with a clay texture and flat relief predominate in the region, with approximate altitude of 750 m.

Fruits and pyrenes morphometric analysis

Fruits of *B. purpurascens* were randomly collected from adult plants for morphometric evaluation. Due to the limited number of mature plants with fruits at the time of collect and the disparity between morphotypes, the proportions varied for each population (Table 1), comprising 69 individuals and 1,380 fruits harvested and processed. We harvest about 20 and 25 fruits directly from the plants, discarding those with any injury, adopting as standard 20 fruits per plant for the morphometric characterization. We sort the fruits in the field, packing them in paper bags, one for each individual, and identified by the yellow and magenta morphs (Figure 1).

Morphometric features evaluated for each fruit were length, width and weight. Then, we pulp the fruits and perform the same measures for the pyrenes (seed + endocarp), individually. Measurement of fresh and dry pulp mass (epicarp + mesocarp) was performed together for all 20

Table 1 - Adult plants number of the yellow and magenta morphs of *Butia purpurascens*, collected in each population (P1, P2, P3 and P4) in the municipality of Jataí, Goiás.

Populations (coordinates)	Yellow morph	Magenta morph	Total
P1 (17° 51'S e 51° 40'O)	3	11	14
P2 (17° 55'S e 51° 42'O)	12	7	19
P3 (17° 49'S e 51° 45'O)	9	10	19
P4 (17° 59'S e 51° 48'O)	13	4	17
Total	37 (740 fruits)	32 (640 fruits)	69 (1380 fruits)

fruits of each plant, following the protocol used by MOURA et al. (2010). Pulps were dehydrated in a kiln closed circulation at 65 °C for 48 hours, to determine the average dry mass, pulp moisture and fruit pulp percentage. The last was obtained by the difference between fresh and dry mass, divided by fresh mass. We use a digital calliper and precision balance for measurements.

Data analysis

We applied the *t* test to compare the morphometric features between the morphs (yellow and magenta) and Analysis of Variance (ANOVA *one way*) for the analyzes among the four populations, using the posteriori Tukey test. The analyzes were performed using the *BioEstat* 5.0 software (AYRES et al., 2007) at 5% significance.

RESULTS AND DISCUSSION

Morphometric features between morphotypes

B. purpurascens fruits showed 6.41 ± 1.87 g weight, 27.49 ± 2.29 mm length and 25.82 ± 2.29 mm width, regardless of the morphotypes and populations evaluated. Pyrene weighed, on average, 1.13 ± 0.58 g and, showed 18.99 ± 1.92 mm length and 10.06 ± 1.24 mm width. The aggregate pulp weight per plant showed 101.51 ± 27.34 g of fresh mass and 23.24 ± 5.61 g of dry mass. The pulp moisture was 76.68 ± 3.35%. Morphometric features of the fruits were similar to the congeneric species *B. capitata* (MOURA et al., 2010; SILVA & SCARIOT, 2013).

We recorded significant differences in all morphometric features between yellow and magenta morphs, except in the percentage of pulp moisture and in the pyrene weight (Figure 2). The yellow morph had greater weight ($t = 5.9$; $p < 0.001$), length ($t = 5.2$, $P < 0.001$) and width ($t = 5.2$; $P < 0.001$) than the magenta morph, beyond the length of the pyrene ($t = 3.0$; $P < 0.01$). Unlike, magenta morph had a larger pyrene width than the yellow morph ($t = 5.2$; $P < 0.001$). Fresh ($t = 2.2$; $P < 0.05$) and dry mass ($t = 2.4$; $P < 0.05$) were significantly higher in the yellow than in the magenta morph.

In addition to the color difference recorded in the other reproductive structures of *B. purpurascens* (GUILHERME et al., 2015), our results showed significant morphometric distinctions in fruits and pyrenes between yellow and magenta morphotypes. Thus, we suggested that there is some genetic difference between them. This may indicate the presence of distinct varieties in *B. purpurascens*, regarding the reproductive structures evaluated. The occurrence of varieties in a species with economic potential can be useful to select lineage with better commercial viability. This can be better applied in the production of seedlings, through the selection of larger pyrenes and/or the production of larger fruits, tastier and more suitable for consumption (PASSOS et al., 2014; PADILHA et al., 2016; SANTOS et al., 2017). In this respect, the yellow morph of *B. purpurascens* would be indicated to obtain a larger amount of pulp.

However, for the selection of pyrenes aiming seedling production, both morphotypes would be indicated, due to the incipient morphometric differences between them. As already reported in other morphometric studies with *B. capitata*, that

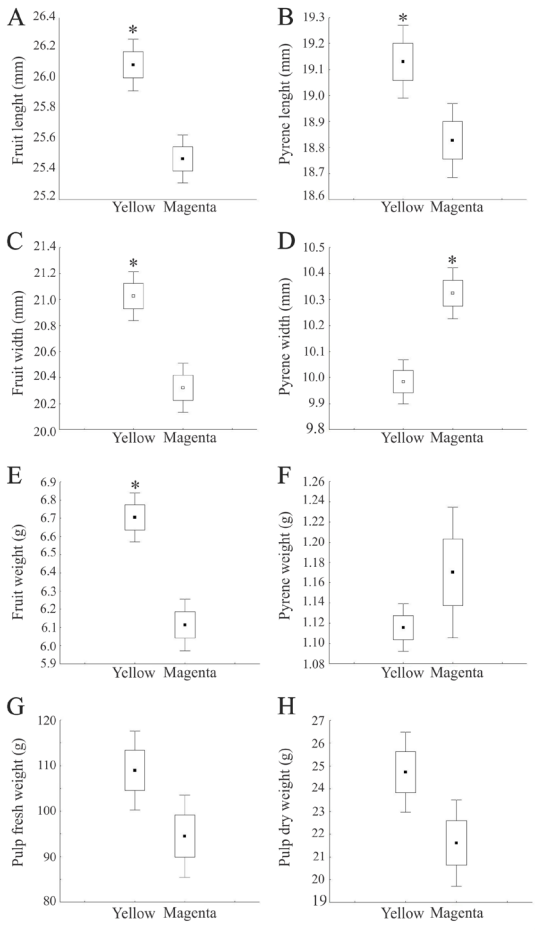

Figure 2 - Morphometric features of fruits and pyrenes between yellow and magenta morphs of *Butia purpurascens*. Asterisks designate significant differences by *t* Test. Error bars denote the standard error

larger pyrenes have more vigor and germination potential and generate healthier seedlings (SILVA & SCARIOT, 2013). In a way, this can be advantageous, considering the maintenance of both morphotypes in future plantings.

Morphometric features between populations

Morphometric variables of fruit and pyrene showed significant differences among the four evaluated populations (Table 2), except for the fruit length. Palms of P4 population showed the highest values for almost all parameters, except for the pyrene width. Fruit weight and width varied by 26.7% and 9.8% among populations, respectively. Pyrene showed variation of 16.0% for weight, 8.3% for length and 4.8% for width. The fresh and dry mass weight varied even more notably, with 24.9% and 27.8%, respectively, among populations. Some factors can act on the size and weight of fruits and pyrenes, as the harvest time and the respective stage of fruit maturity, besides abiotic aspects such as edaphic conditions, light incidence and microclimate under which each plant grows (FELIZARDO et al., 2015).

However, the degree of fragmentation of *B. purpurascens* populations, with an anthropized landscape due to agricultural activities and proximity to the Jataí city urban perimeter (GUILHERME & OLIVEIRA, 2011), may be responsible for the recorded differences and the isolation process among them. Over time, this fragmented matrix can limit the performance of potential pollinators, especially invertebrates and seed dispersers (XIAO et al., 2016), causing disruption in gene flow between populations. AGUILAR et al. (2019) reported gene flow and reproduction mode as essential factors for the genetic structure of plant populations. Therefore, due to reproductive morphometric differences, we suggested that fragmentation can cause these effects in the long-term. Other native, commercial and ecologically important plant species from the Cerrado showed morphometric differences between studied populations, as is the case of *Dipteryx alata* Vogel (BOTEZZELLI et al., 2000), *Harconia speciosa* Gomes (GANGA et al., 2010), *Mauritia flexuosa* (MATOS et al., 2014) and *Annona crassiflora* (PALERMO & SOUZA, 2019). This may indicate isolation of populations, caused by habitat loss and fragmentation.

Therefore, the natural ecosystems degradation can trigger the occurrence of genetic disruption, depending on the population density of the species (XIAO et al., 2016; BROWNE & KARUBIAN, 2017). In our study, differences in the morphometric features recorded between the populations of *B. purpurascens*, indicated particularities between the sites, even if geographically close. This suggested that regional anthropic pressures have provided isolation for plant populations and the consequent lack of gene flow. In order to conserve *B. purpurascens*, is essential and urgent to deepen the knowledge about genetic diversity and increase the efficiency in its propagation. This is because recent studies point to high seed predation and low germination potential (RESSEL & GUILHERME, 2022), added to other factors, which leads the species to be critically endangered status (CNCFLORA, 2020).

Table 2 - Morphometric features of fruits and pyrenes of *Butia purpurascens*, compared between four populations (P1, P2, P3 and P4) by ANOVA. Different letters designate significant differences by Tukey test. FM: fresh pulp mass; DM: dry pulp mass. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Features	P1	P2	P3	P4	ANOVA
Fruit weight (g)	6.19±1.65 b	5.80±1.83 c	6.21±1.80 b	7.46±1.78 a	70.31***
Pyrene weight (g)	1.12±0.99 b	1.05±0.35 b	1.11±0.32 b	1.25±0.36 a	8.27***
Fruit width (mm)	20.23±2.33 b	19.76±2.30 c	20.65±2.50 b	21.87±2.56 a	20.48***
Fruit length (mm)	25.40±2.43	25.83±2.59	25.26±2.08	26.77±1.65	0.81
Pyrene width (mm)	9.72±1.21 a	10.21±1.36 b	10.21±1.14 b	10.10±1.18 b	14.65***
Pyrene length (mm)	18.79±2.01 b	18.74±2.10 b	18.39±1.58 c	20.05±1.51 a	64.51***
Weight FM (g)	95.18±24.03 c	93.41±22.26 c	99.28±28.86 b	118.18±28.16 a	3.83*
Weight DM (g)	21.39±3.96 b	19.77±4.24 b	24.39±5.58 a	27.39±5.52 a	9.52***
% moisture	76.90±3.4 b	78.4±3.30 b	74.90±3.50 a	76.50± 2.20 b	4.37*

CONCLUSION

In addition to different colors in the reproductive structures, the *B. purpurascens* morphotypes showed significant differences in the morphometric features of fruits and pyrenes. Yellow morph showed larger, heavier and pulpier fruits than the magenta morph. Therefore, the yellow morph is commercially more viable; although, both morphotypes are recommended for propagation studies.

Significant morphometric differences were also observed among the four populations, suggesting that the fragmentation of natural ecosystems can interrupt the gene flow between populations, and cause deleterious genetic effects in *B. purpurascens* in long-term.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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