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PROGRAMA DE PÓS-GRADUÇÃO *Stricto Sensu* EM CIÊNCIAS
AGRÁRIAS - AGRONOMIA

PARÂMETROS GENÉTICOS E DIVERSIDADE EM TESTE DE PROGÊNIES E PROCEDÊNCIAS DE BARUEIRO MEDIANTE CARACTERÍSTICAS MORFOLÓGICAS E FISIOLÓGICAS

Autora: Laísse Danielle Pereira
Orientador: Dr. Fernando Higino de Lima e Silva

Rio Verde - GO
Dezembro-2022

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Autora: Laísse Danielle Pereira
Orientador: Dr. Fernando Higino de Lima e Silva

TITULAÇÃO: Doutorado em Ciências Agrárias-Agronomia, Área de Concentração em Produção Vegetal Sustentável no Cerrado

APROVADO em, 15 de dezembro de 2022.

Prof. Dr. Fernando Higino de Lima e Silva (Presidente)

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Prof. Dr. Charlys Roweder (Avaliador externo)

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BIOGRAFIA DA AUTORA

Nascida no dia 02 de agosto de 1993, na cidade de Petrolina de Goiás–GO, filha de Moisés Pereira Neto e Ana Maria Lino Teixeira Pereira. Ingressou no curso de Agronomia no Centro Universitário de Anápolis – UniEvangélica, Anápolis-GO, no mês de fevereiro de 2011 e obteve o título de Engenheira Agrônoma em abril de 2016. Em agosto de 2016 iniciou no Mestrado em Agronomia, área de concentração Produção Vegetal, no Programa de Pós-Graduação em Agronomia da Universidade Federal de Goiás - Regional Jataí, Jataí-GO, sob orientação da Profª Drª Danielle Fabíola Pereira da Silva, obtendo o título de Mestra em julho de 2018. Em setembro de 2018 ingressou no Doutorado em Ciências Agrárias – Agronomia, área de concentração Produção Vegetal Sustentável no Cerrado, no Programa de Pós-Graduação em Ciências Agrárias – Agronomia do Instituto Federal Goiano – campus Rio Verde, Rio Verde-GO, sob orientação do Prof. Dr. Fernando Higino de Lima e Silva, obtendo o título de Doutora em dezembro de 2022.

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CAPÍTULO II

LISTA DE SIMBOLOS, SIGLAS, ABREVIAÇÕES E UNIDADES

BLUP	- *Best Linear Unbiased Predictor*
REML	- *Restricted Maximum Likehood*
UPGMA	- *Unweighted pari-group method using an arithmetic average*
SD	- Stem diameters
PH	- plant height
cm	- centímetros
mm	- milímetros
Chl a	- índice de clorofila "a"
Chl b	- índice de clorofila "b"
Chl t	- índice de clorofila total
F_V/F_M	- Photosystem II photochemical efficiency
ET_0/RC	- electron transport per reaction center
DI_0/RC	- antenna chlorophyll level specific energy dissipation flux
$PhiD_0$	- dissipation energy efficiency
Pi_{ABS}	- energy conservation performance index from Photosystem II-absorbed photons
σ_f^2	- Variância fenotípica
σ_a^2	- Variância aditiva
σ_{prov}^2	- Variância de procedência
σ_e^2	- Variância de erro

RESUMO

PEREIRA, LAÍSSE DANIELLE. Instituto Federal Goiano – campus Rio Verde – GO, outubro de 2022. **Parâmetros genéticos e diversidade em teste de progênies e procedências de barueiro mediante características morfológicas e fisiológicas.** Orientador: Prof. Dr. Fernando Higino de Lima e Silva. Coorientadores: Drª Danielle Fabiola Pereira da Silva e Dr. Pablo Diego Silva Cabral.

Objetivou-se com este trabalho fornecer fundamentos e suporte para ações de uso e conservação do barueiro avaliando características de desenvolvimento inicial em um teste de progênie e procedências. Este trabalho encontra-se dividido em dois capítulos: o primeiro, objetiva estimar parâmetros genéticos para caracteres de crescimento, enquanto o segundo objetiva estimar a diversidade genética entre progênies por meio do uso combinado de caracteres morfológicos e fisiológicos, além de determinar quais características mais contribuem para diferenciação das progênies. O trabalho foi conduzido no banco de germoplasma "*ex situ*" de barueiro instalado no Instituto Federal Goiano – campus Rio Verde, sendo constituído por 31 progênies de meios-irmãos advindas de quatro procedências no estado de Goiás, a saber: Bom Jardim de Goiás, Ipameri, Iporá e Urutaí. As progênies foram avaliadas quanto a caracteres morfológicos (altura de planta e diâmetro do caule) e fisiológicos (índices de clorofila e parâmetros fotoquímicos). Para a estimativa dos parâmetros genéticos os caracteres foram analisados por meio da metodologia de modelos mistos - procedimento REML (Máxima Verossimilhança Restrita) / BLUP (Melhor Predição Linear não Viesada). Para a estimativa de diversidade genética, os dados foram analisados através da importância para a diversidade e posteriormente agrupados através de dendrograma UPGMA utilizando a distância generalizada de *Mahalanobi*s. Os caracteres fotoquímicos demonstraram ser mais influenciados pelo ambiente do que os caracteres morfológicos, apresentando baixa magnitude e grande variação das estimativas de herdabilidade ao longo do ano. Entretanto, apesar dos caracteres fotoquímicos serem respostas de estresse ambiental, eles expressaram-se de forma diferente entre si e entre os índices de clorofila. Os caracteres que mais contribuíram para a discriminação das progênies foram os índices de clorofila em ambas as estações. Os resultados obtidos com a estimativa de parâmetros genéticos e quantificação da divergência genética entre as progênies sob estudo servirão de subsídio para futuras estratégias de manejo e conservação da própria espécie e poderão servir de modelo para outras espécies.

Palavras-chave: Cerrado; *Dipteryx alata* V.; delineamento genético; modelos mistos; REML/BLUP; UPGMA.

ABSTRACT

PEREIRA, LAÍSSE DANIELLE. **GENETIC PARAMETERS AND DIVERSITY AT PROGENY AND PROVENANCE TEST OF 'BARUEIRO' THROUGH MORPHOLOGIC AND PHYSIOLOGIC CHARACTERS. 2022.** Thesis (doctorate in Agrarian Science) – Federal Institute Goiano – campus Rio Verde, Rio Verde-GO. Advisor: Dr. Fernando Higino de Lima e Silva

The objective of this work was to provide fundamentals and support for actions of use and conservation of baru tree, evaluating characteristics of initial development in a test of progeny and provenances. This work is divided into two chapters, the first one aims to estimate genetic parameters for growth traits, while the second one aims to estimate the genetic diversity betwen progenies through the combined use of morphological and physiological characters, in addition to determine which characteristics most contribute to the progenies differentiation. The work was carried out in the *ex-situ* barueiro germplasm bank installed at the Instituto Federal Goiano – Rio Verde campus, consisting of 31 half-sibling progenies from four origins in the state of Goiás. Namely: Bom Jardim de Goiás, Ipameri, Iporá and Urutaí. The progenies were evaluated for morphological (plant height and stem diameter) and physiological (chlorophyll index and photochemical parameters) characters. To estimate the genetic parameters, the characters were analyzed using the mixed model methodology - REML (Restricted Maximum Likelihood) / BLUP (Best Linear Unbiased Prediction) procedure. For genetic diversity estimation, the data were analyzed according to their importance to diversity and then clustered through the UPGMA dendrogram using the generalized Mahalanobis distance. Photochemical characters proved to be more influenced by the environment than morphological characters, showing low magnitude and great variation of heritability estimates throughout the year. However, despite the photochemical characters being environmental stress responses, they were expressed differently among themselves and between chlorophyll indices. The characters that most contributed to the discrimination of the progenies were the chlorophyll index in both seasons. These results obtained with the genetic parameters estimation and quantification of the genetic divergence between the progenies under study will serve as a basis for future management and conservation strategies for the species itself and may serve as a model for other species.

Keywords: Cerrado; *Dipteryx alata* V; genetic delimitation; mix models; REML/BLUP; UPGMA.

INTRODUÇÃO

O Brasil contempla grande diversidade contida nas suas formações vegetativas (biomas), dentre elas, o Cerrado configura como a segunda maior (SILVA, *et al*., 2008) e que vem sofrendo com a exploração intensa e desordenada das florestas remanescentes, provocando erosão genética de espécies florestais de reconhecido valor econômico (MENDES & PAULA, 2015). Dentre as diversas espécies florestais, as frutíferas têm se destacado, pois constituem importante fonte alimentar e de renda (VERA *et al*., 2009).

A espécie *Dipteryx alata* Vogel apresenta grande relevância entre as frutíferas encontradas no Cerrado, por sua grande aceitação pela qualidade de sua amêndoa e pelos múltiplos usos que a espécie permite (SILVA *et al*., 2021). Como não há disponibilidade de sementes selecionadas comerciais, o produtor deveria iniciar o plantio a partir da coleta de frutos no campo, entretanto, até o momento, sua exploração é feita majoritariamente via extrativismo (PEREIRA & PASQUALETO, 2011; SILVA *et al*., 2021). Esta prática é problemática a médio e longo prazo (COLEVATTI *et al*., 2010), levando diversas entidades a abraçar o problema, estabelecendo programas de conservação de recursos genéticos.

Para que haja utilização prática dos recursos genéticos, e quando instalado, ampliar a base de programa de melhoramento, as atividades de pré-melhoramento são de grande importância (LOPES *et al*., 2011). Essas atividades envolvem a identificação de genes e/ou características de interesse em bancos de germoplasma.

A instalação de banco de germoplasma, seguindo o delineamento genético de teste de progênies e procedências, atende várias finalidades importantes para a conservação, como conservar a variabilidade genética da população natural, viabilizar a caracterização e formar pomares de sementes por mudas (SEBBENN *et al*., 2007). Por ser um plantio sistematizado, permite que ao longo do tempo possam ser estimados parâmetros genéticos e, quando conveniente, os ganhos de seleção, assim sendo, esses materiais genéticos são conservados e podem ser melhorados (CANUTO *et al*., 2015). Ou seja, a formação do teste de progênies de *Dipteryx alata* contribui para o resgate de material genético, com variabilidade genética suficiente para garantir a formação de novas populações.

A caracterização dessas progênies deve ser realizada a fim de possibilitar a utilização pelos melhoristas na obtenção de indivíduos com potencial para o posterior desenvolvimento de cultivares (ARAÚJO *et al*., 2019). Para que haja melhor entendimento do material disponível, a caracterização pode ser feita abrangendo as mais diferentes faces da planta: morfológica, fisiológica, molecular etc. Quanto mais completa a caracterização, maior será o respaldado para o melhorista tomar decisões quanto à escolha de genitores e o planejamento de possíveis cruzamentos.

A avaliação das propriedades genéticas das características de crescimento nos primeiros anos de desenvolvimento é de grande utilidade para o melhorista que objetiva, além da identificação dos melhores indivíduos, progênies e procedências, a quantificação da eficiência da seleção precoce (GIORDANI *et al*., 2012). Com a identificação de características das árvores em idade juvenil que estejam relacionadas com aquelas de interesse econômico em indivíduo adulto, pode-se diminuir o tempo para completar um ciclo de seleção, o que resulta em maior ganho genético por unidade de tempo (MASSARO *et al*., 2010).

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OBJETIVOS

2.1 Objetivo Geral

Fornecer informações para ações de conservação genética do barueiro mediante caracterização em estádio de crescimento inicial em um teste de progênie e procedências.

2.2 Objetivos Específicos

- Avaliar caracteres morfológicos e fisiológicos;
- Estimar parâmetros genéticos para cada caractere via REML;
- Estimar a diversidade genética das progênies por meio do uso combinado de caracteres morfológicos e fisiológicos;
- Determinar quais características mais contribuem para diferenciação das progênies.

CAPÍTULO I

(Normas de acordo com a revista *European Journal of Forest Research*)

Genetic parameters in *Dipteryx alata* Vogel employing a progeny and provenance test in the Brazilian Savanna: a morphological and physiological approach assessing different seasons.

Abstract

Dipterix alata Vogel, commonly known as barueiro, has increasingly suffered genetic erosion due to the extractive exploitation of its fruits. Progeny and provenance tests are paramount as a conservation strategy, allowing for estimates on different genetic trait parameters over time. Due to the importance of this species conservation and the absence of studies on its behavior considering seasonal variations, this study aimed to estimate genetic parameters via the REML/BLUP methodology, in initial barueiro stages through morphological, chlorophyll and photochemical analyses. Progenies obtained in February 2018 from the *ex-situ* barueiro germplasm bank located at the Federal Goiano Institute, Rio Verde campus, Rio Verde, in the state of Goiás, Brazil, were, therefore, assessed in this regard. The progeny and provenance test comprised 31 half-sibling progenies generated from seeds obtained from matrices located in four Goiás municipalities, namely Bom Jardim de Goiás, Ipameri, Iporá and Urutaí. The progenies were allocated in a randomized block design consisting of 10 replications and one plant per experimental plot. Photochemical characters were highly influenced by the environment compared to morphological characters, displaying low magnitude and high heritability estimate variations throughout the year. However, even though the investigated photochemical traits comprise environmental stress responses, differential expressions were noted both amongst themselves and among chlorophyll content traits. The higher genetic variability at the individual level noted for all investigated traits, however, indicates the possibility of satisfactory genetic gains for superior progenies selection.

Keywords Barueiro · Chlorophyll a · Fluorescence · Heritability · REML/BLUP

3.1 Introduction

Dipteryx alata Vogel (Fabaceae family), also known as barueiro, baruzeiro and cumbaru, is a fruit tree species endemic to the Brazilian Savannah. Due to the economic, nutritional, and medicinal values of its nuts (Oliveira-Alves et al. 2020), barueiro trees present high potential for use in both agroforestry systems and commercial orchards. Barueiro fruit extraction has, however, contributed to the genetic erosion of the species and low natural perpetuation combined with perpetuation of inferior genetic material (Silva et al. 2016). To prevent this process from continuing, conservation strategies allied to genetic variability preservation methods and exploitation are required. In this regard, e*x-situ* germplasm banks established through progeny and provenance tests are an interesting alternative, allowing the monitoring of different individual phenotypic and genotypic traits over time to be used as the base population of breeding programs (Zaruma et al. 2015).

Obtaining good development genotypes from parents that allow this inference demonstrates the importance of applying genetic parameter estimates in each population (Costa et al. 2015; Nogueira et al. 2019). Genetic parameters estimated through mixed models employing the Restricted Maximum Likelihood/Best Unbiased Linear Prediction (REML/BLUP) methodology allow the prediction of intra- and interpopulation genetic values and genetic gain maximization through selection choices (Pimentel et al. 2014).

Despite comprising a typical Brazilian Savannah species adapted to this biome’s conditions, barueiro trees can suffer from the accentuated climatic seasonality of this biome (Penereiro et al. 2018). Plants acclimatized to this type of environment tend to develop certain mechanisms to minimize photosynthetic damage, including photoinhibitory processes (Gonçalves et al. 2010), which directly affect plant growth and development. In certain regions, such as the Savanna, where water scarcity periods combined with high temperatures are usual, plant stress toleration abilities become important features, as loss of photochemical efficiency may take place, resulting in a series of physiological processes that culminate in decreased plant growth or even death. Chlorophyll fluorescence parameter evaluations are employed in this regard, providing qualitative and quantitative light energy absorption and performance data through the photosystem II (Schock et al. 2014), furthering knowledge on potential physiological disturbances (Santos et al. 2019).

Studies conducted on several species (Schock et al. 2014; Peloso et al. 2017; Rodrigues et al. 2017) demonstrate the importance of these measures for plant cultivation, although most of them note that photosynthetic performance assessments usually provide only phenotypic information. The use of information obtained at the genetic level conveys more robust genotype information for use in pre-breeding programs and, consequently, greater knowledge on conservation processes. According to Mezzomo et al. (2021), physiological trait evaluations alongside agronomic appraisals are promising for genetic parameters studies employing the REML/BLUP methodology.

In this context, this study aimed to evaluate the influence of seasonal variations on genetic barueiro parameter estimates in a progeny and provenance test carried out in early barueiro stages in the Brazilian Savannah based on morphological, chlorophyll and photochemical characters.

3.2 Material and methods

Germplasm bank

The progeny and provenance test (*ex-situ* barueiro germplasm bank (*Dipteryx alata* Vogel)) was conducted in the field at the Federal Goiano Institute, Rio Verde campus (17º48'36"S, 50º54'10"W) in the state of Goiás, Brazil, on February 23, 2018 (Fig. 1). The genetic germplasm bank material is composed of 31 half-sib progenies generated from seeds from four provenances in Goiás, namely Bom Jardim de Goiás, Ipameri, Iporá and Urutaí. The progenies were allocated in a randomized block design consisting of 10 repetitions, one plant per experimental plot, in a 5 x 4 m spacing, totaling 310 plants.

Barueiro progenies were submitted to morphological and physiological assessments (chlorophyll index and photochemical characters) at 16 months after transplanting, beginning in the winter of 2019 and ending in the fall of 2020. The beginnings of each experimental seasonal period were 06/21/2019 (Winter), 09/23/2019 (Spring), 12/22/ 2019 (Summer) and 03/20/2020 (Autumn).

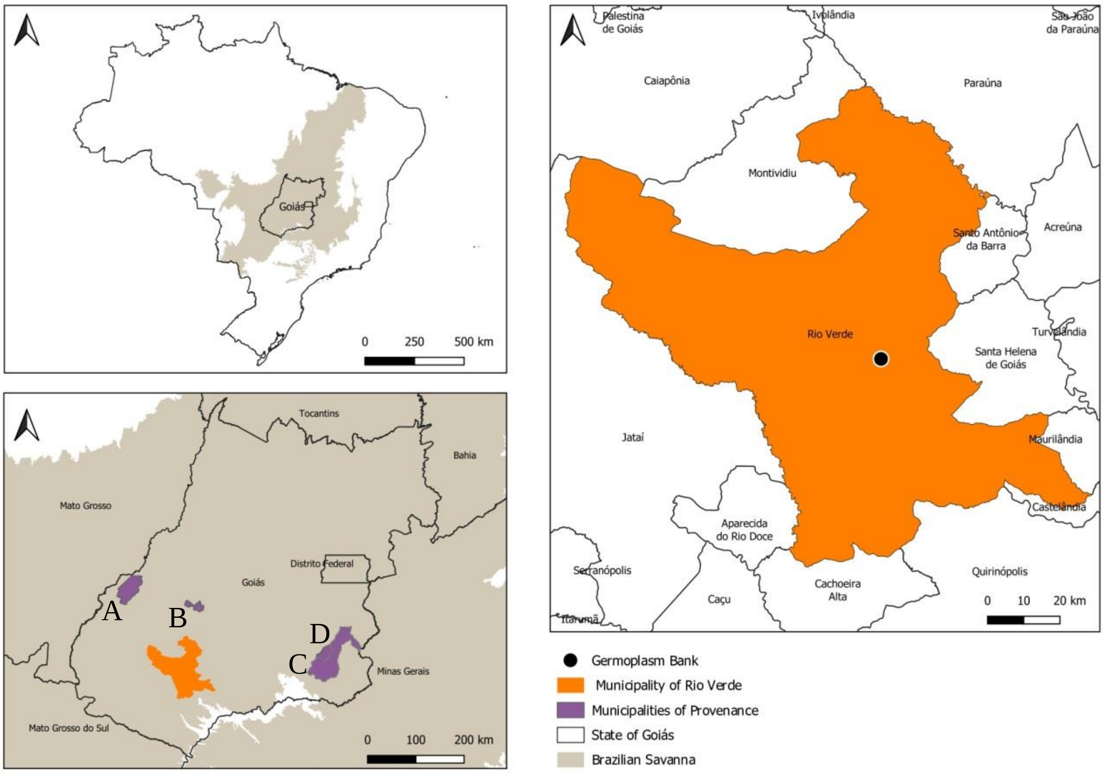

Fig. 1 Brazilian Savanna distribution, highlighting the state of Goiás, the progeny municipalities (A: Bom Jardim de Goiás, B: Iporá, C: Ipameri, D: Urutaí) and the municipality (Rio Verde) where the experiment was implemented.

The average temperature and rainfall rates during the experimental period are depicted in Fig. 2.

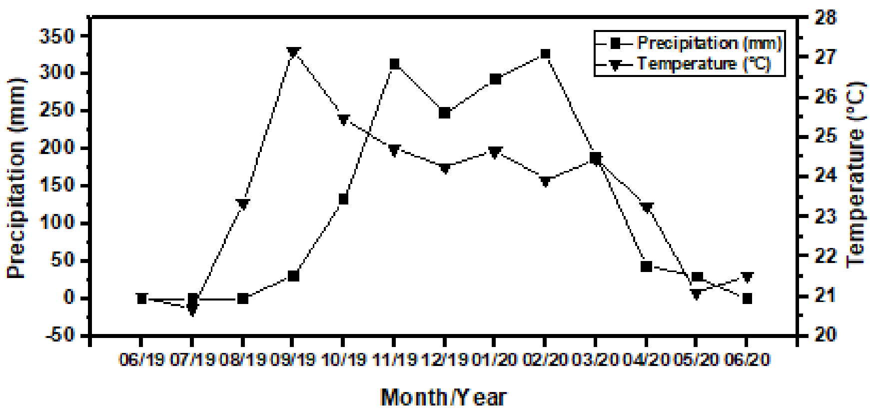

Fig. 2 Average temperature and rainfall rates during the experiment period (06/01/2019 to 06/30/2020) in the municipality of Rio Verde, Goiás, Brazil. Source: Inmet (2021) and Agritempo (2021).

Morphological characters

Stem diameters (SD) were measured 2 cm from the ground with a digital caliper (mm) and plant height (PH) was measured from the ground to the insertion of a new leaf in the apical meristem of the main stem with a graduated ruler (cm). Observations consisted of the average of five evaluations at equally distributed in intervals within each season of the year.

Physiological characters

Chlorophyll index

Chlorophyll a and b indices were evaluated employing an electronic CFL 1030 ClorofiLOG chlorophyll meter (Falker Automação Agrícola, Brazil) to obtain Falker Chlorophyll Index values. The measurements were performed in the middle third of the leaf blade of the 3rd or 4th completely expanded leaf which had not yet begun the senescence process. Chlorophyll a (Chl a) and chlorophyll b (Chl b) indices were determined, and the total chlorophyll indices (Chl t) and chlorophyll a/chlorophyll b ratio (Chl a/Chl b) were calculated. Observations consisted of the average of five evaluations at equally distributed intervals within each season of the year.

Photochemical characters

Chlorophyll a fluorescence was evaluated employing an F100 FluorPen fluorometer (Photon systems Instruments). Measurements were performed on completely expanded healthy and dark-adapted leaves for 30 minutes with the aid of tweezers. The parameters established by the JIP-Test were estimated based on fluorescence intensities through a chlorophyll a fluorescence analysis, comprising the following parameters: Photosystem II photochemical efficiency (F_V/F_M), electron transport per reaction center (ET_0/RC), antenna chlorophyll level specific energy dissipation flux (DI_0/RC), dissipation energy efficiency ($PhiD_0$) and energy conservation performance index from Photosystem II-absorbed photons (Pi_{ABS}). Observations consisted of only one evaluation for all individuals at the end of each season, due to logistic reasons.

Statistical genetic analyses

Variance components were estimated using the restricted maximum likelihood method (REML). The SELEGEN-REML/BLUP software (Resende 2016) was used for the trait analyses employing the model 5, recommended for complete block experiments in one location and the evaluation of several half-sib progeny populations:

$$y = Xr + Za + Wp + Ts + e$$

Where y is the data vector, r is the repetition effect vector (assumed to be fixed) summed to the overall mean, a is the individual additive genetic effect vector (assumed to be random), p is the plot effect vector (assumed to be random, not estimated herein due to the presence of only one plant per plot), s is the population or provenance effect vector (assumed to be random) and e is the error or residual vector (random). Capital letters represent the incidence matrices for the investigated effects.

The estimated variance components were as follows: individual additive genetic variance (σ_a^2), additive genetic variance between provenances ($\hat{\sigma}_{prov}^2$), residual variance ($\hat{\sigma}_e^2$) and individual phenotypic variance (σ_f^2), used to determine the individual heritability in the narrow sense, coefficient of genotypic variation between progenies, residual variation coefficient and $\hat{m}$ (general trait average). The results were plotted as graphs using the Origin software.

3.3 Results and discussion

Morphological characters

The average phenotypic plant height (PH) and stem diameter (SD) values for the 31 evaluated half-sib progenies are depicted in Fig. 3. A PH variation of 61.34 in the winter to 109.50 cm in autumn was observed, representing a 48.16 cm/year increase, while SD ranged from 17.62 cm in winter to 28.55 cm in autumn, representing a 10.93 cm/year increment. According to Pagliarini et al. (2016), the expected performances for these morphological *Dipteryx alata* population traits are slow, like other native Brazilian species presenting higher wood density.

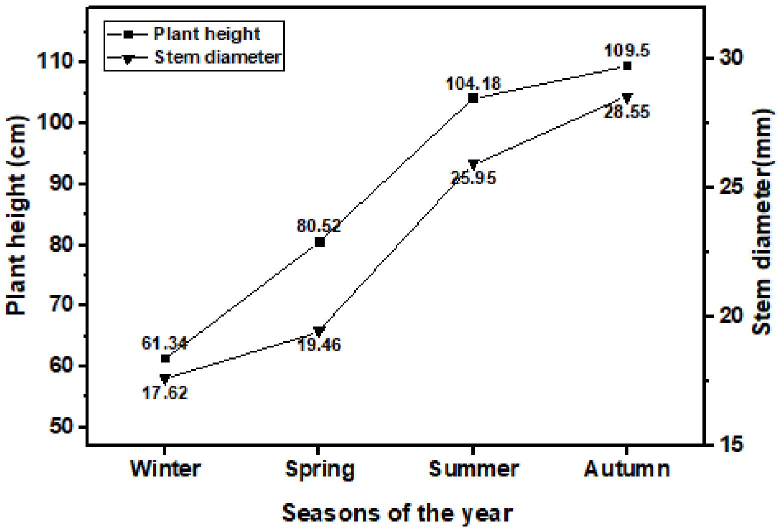

Fig. 3 General morphological trait averages in each evaluated season. (Rio Verde–GO, 2019-2020).

The estimated variance components for each morphological trait are presented in Fig. 4.

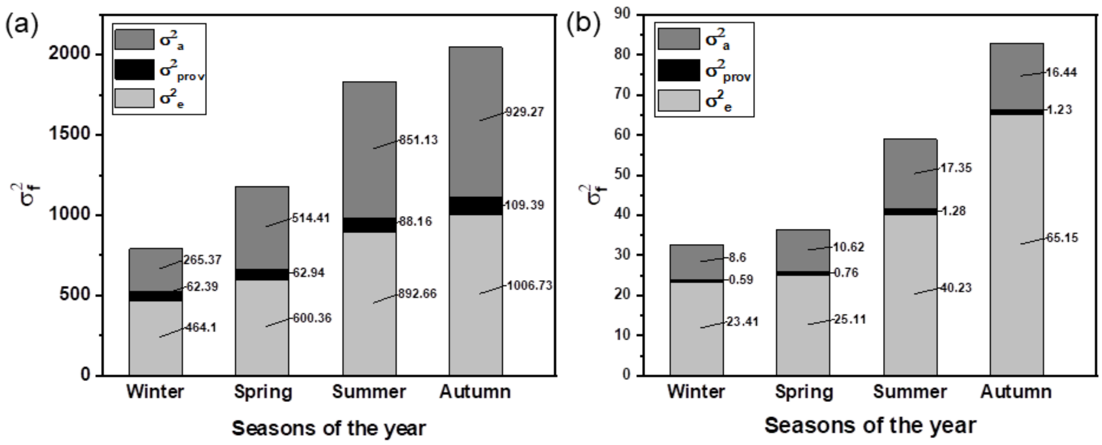

Fig. 4 Variance components for **a** plant height (PH) and **b** stem diameter (SD). σ_f^2: phenotypic variance; σ_a^2: additive variance; σ_{prov}^2: provenance variance; and σ_e^2: error variance.

Estimated σ_f^2 $(\sigma_a^2 + \sigma_{prov}^2 + \sigma_e^2)$ values increased throughout the seasons for PH and SD. Concerning PH (Fig. 4a), similar behavior was observed for all variances employed in the σ_f^2 equation, while slight decreases in SD σ_a^2 and σ_{prov}^2 were observed in the autumn (Fig. 4b) compared to summer.

Narrow sense heritability (h_a^2) values for each morphological trait are presented in Fig. 5, ranging from 0.33 (winter) to 0.46 (summer) for PH and from 0.20 (autumn) to 0.29 (summer) for SD. According to Resende and Alves (2020) h_a^2 the magnitudes are categorized as very high (≥ 0.8), high (0.5 – 0.8) moderate (0.15 – 0.5) or low (0.01 – 0.15). Therefore, both traits investigated herein were moderate during all season.

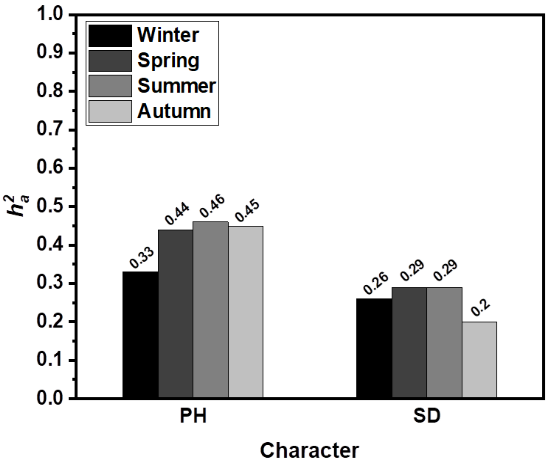

Fig. 5 Narrow sense heritability (h_a^2) values for plant height (PH) and stem diameter (SD).

The two morphological traits investigated herein displayed increased h_a^2 estimateds from winter to summer, with a decrease noted in autumn (Fig. 5). PH suffered greater environmental influence in the winter, while SD suffered in autumn. It is important to note that autumn represents the beginning of the dry period and, consequently, lower plant growth, when responses associated to environmental control over PH begin. These responses may be more expressive or occur faster for certain traits, which could explain the observed difference in estimates, given that the summer takes place before autumn.

Canuto et al. (2015), in a study in the state of São Paulo with barueiro progenies in a progeny and provenance test, implemented during three different plant growth stages, reported 0.80, 0.38, 0.25 h_a^2 PH estimates at three, seven and 21 months of age, respectively. However, the 21-month-old plants investigated in the present study correspond to spring, with the observed value (0.44) higher than that reported by Canuto et al. In another assessment, Andrade et al. (2020), reported a PH h_a^2 estimate of 0.38 in a progeny test in the state of Mato Grosso with 30-month-old plants. Additionally, values lower than those observed in the present study were reported by Zaruma et al. (2015) in a barueiro progeny and provenance test in the state of Mato Grosso do Sul, of 0.19, in nine-year-old progenies, while Pagliarini et al. (2016) reported an h_a^2 estimate of 0.25 in a test on 25-year-old barueiro progenies in the state of São Paulo. These differences demonstrate how much h_a^2 estimates can vary for the same trait and species at different ages and in different locations, as h_a^2 is a property of not only one trait, but also of the studied population and environmental circumstances to which individuals are subjected to (Silva et al. 2018).

Given the greater PH heritability observed herein, the genetic control of this trait is greater than for SD, corroborating Pagliarini et al. (2016), who concluded that PH displays greater genetic control when compared to diameter at breast height, wood volume, survival and bifurcation when evaluating 25-year-old barueiro trees employing different growth traits.

The coefficient of variation estimates for the investigated morphological characters are presented in Table 1.

Table 1 Coefficients of variation for plant height (PH) and stem diameter (SD)

Parameters	Characters							
	PH				SD			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
VCgi (%)	26.56	28.17	28.00	27.84	16.64	16.745	16.05	14.20
VCgp (%)	13.28	14.08	14.00	13.92	08.32	08.37	08.03	07.10
VCe (%)	41.98	39.00	37.56	37.69	31.01	29.55	28.12	30.83

VCig%: Individual genetic variation coefficient; VCpg%: Progeny genetic variation coefficient; VCe: Error variation coefficient.

The PH trait presented VCig estimates between 26.56 (winter) and 28.17% (spring), close to those reported by Canuto et al. (2015) when evaluating barureiro progenies at three (39.88%), seven (28.33%) and 21 months (26.93%) of age, of 39.88, 28.33 and 26.93%, respectively. For SD, the authors reported an estimate of 14.93% at seven months, close to that calculated herein, which ranged from 14.20 to 16.75%. VCig estimations are paramount in conservation and improvement programs, as the higher is the VCig value, the greater the possibility of identifying superior individuals that may provide selection gains. Among morphological characters, PH presented higher estimates compared to SD, indicating greater gains obtained from PH selection compared to SD.

Physiological characters

Chlorophyll index

The average phenotypic values of chlorophyll index-related traits, Chlorophyll a (Chl a), Chlorophyll b (Chl b), total chlorophyll (Chl t) and the chlorophyll a/chlorophyll b (Chl a/Chl b) ratio for the 31 evaluated half-sib progenies are displayed in Fig. 6. Changes in the levels/indices of these pigments are associated to imbalances between their synthesis and their degradation, the latter caused by photooxidative stress.

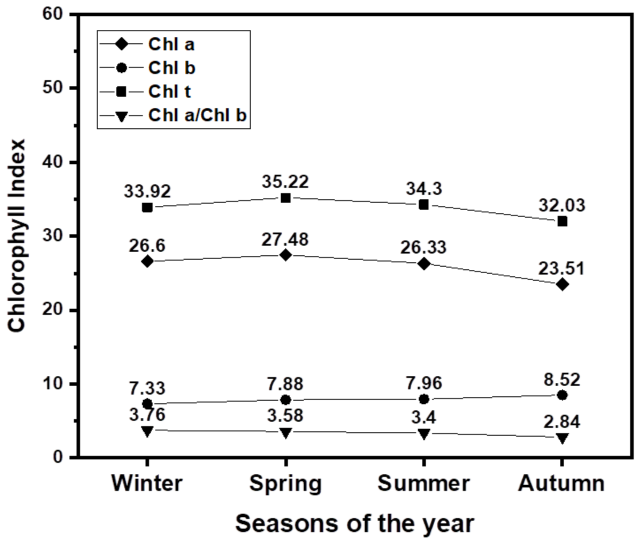

Fig. 6 General average phenotypic values of chlorophyll index-related traits for each season of the year. (Rio Verde-GO, 2019-2020).

The variance components estimated for the chlorophyll index traits are presented in Fig. 7. The σ_f^2 estimates observed for Chl a, Chl t and chl a/chl b were similar, with higher estimates noted in winter, followed by summer, spring and autumn. However, the genetic portion (σ_a^2 + σ_{prov}^2) estimates were higher than σ_e^2 for Chl a, Chl b and Chl t, demonstrating greater genetic control in the spring and autumn. The chl a/chl b ratio (Fig 7d) presented a greater environmental influence on σ_f^2, as σ_e^2 estimates were higher than the σ_a^2 and σ_{prov}^2 estimates for all seasons.

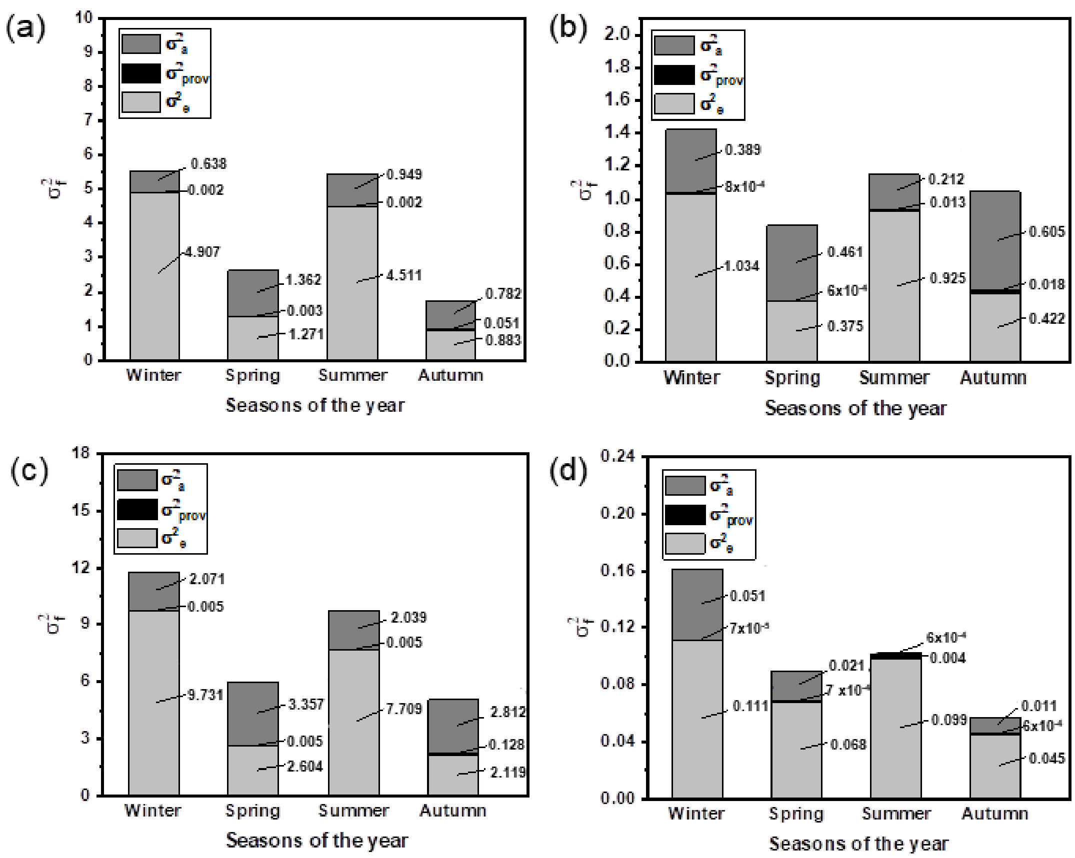

Fig. 7 Variance components for **a** chlorophyll a index (Chl a), **b** chlorophyll b index (Chl b), **c** total chlorophyll index (Chl t), **d** chlorophyll a/chlorophyll b ratio (Chl a/Chl b). σ_f^2: phenotypic variance; σ_a^2: additive variance; σ_{prov}^2: provenance variance; and σ_e^2: error variance.

The h_a^2 estimates for chlorophyll index-associated traits are displayed in Fig. 8. Chl a, Chl b and Chl t displayed similar behavior throughout the year, with high h_a^2 magnitude estimates (Resende and Alves 2020) in the spring and autumn, due to the greater portion of σ_f^2 being attributed to σ_a^2 (Fig. 7a-c), *i.e.*, the observed estimate presents less influence than the environment. Chl a/Chl b, on the other hand, presented a small decrease from winter (0.31) to spring (0.23). In the summer, a much lower h_a^2 estimate (0.01) was noted compared to all other seasons, while the Chl a/Chl b ratio increased (0.20) to a value close to values observed in the first two seasons in autumn.

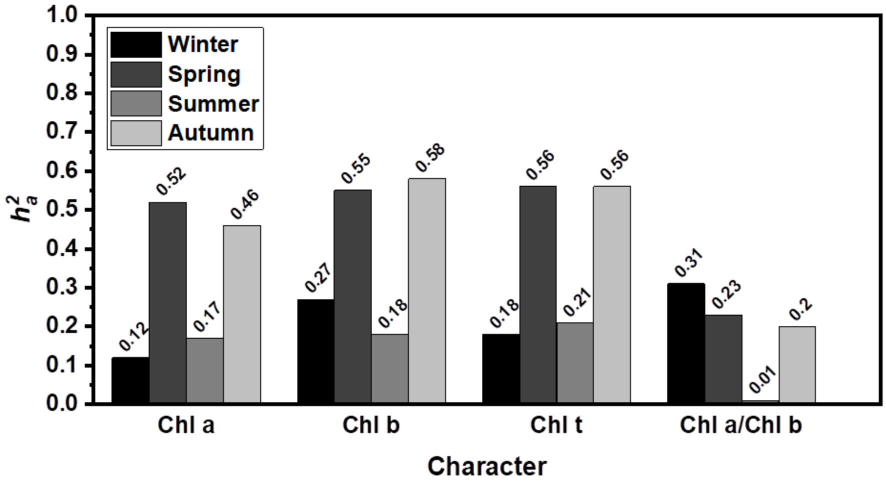

Fig. 8 Narrow sense heritability (h_a^2) for chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl t) and the Chlorophyll a/Chlorophyll b ratio (Chl a/Chl b).

Considering that spring and autumn comprise transition seasons between the most critical climatic seasonality periods, the higher h_a^2 estimates for chlorophyll indices observed herein can be explained by the lower stress that plants suffer during this period, resulting in a greater influence of genetic factors and, consequently, lower environmental influence. Despite the observed h_a^2, variations, the general average did not indicate ample seasonal variations (Fig. 6). On the other hand, all chlorophyll index-associated traits presented low CVgi (Table 2), indicating that the evaluated baureiro plants exhibit low pigment index variability.

Table 2 Coefficients of variation for the chlorophyll an index (Chl a), chlorophyll b index (Chl b), total chlorophyll index (Chl t) and chlorophyll a/chlorophyll b ratio (Chl a/Chl b)

	Characters							
Parameters	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
	Chl a				Chl b			
VCig (%)	3.00	4.25	3.70	3.76	8.51	8.62	5.78	9.13
VCpg (%)	1.50	2.12	1.85	1.88	4.26	4.31	2.89	4.57
VCe (%)	8.73	5.51	8.68	5.15	15.71	10.78	13.08	10.99
	Chl t				Chl a/Chl b			
VCig (%)	4.24	5.20	4.16	5.24	5.99	4.04	0.74	3.76
VCpg (%)	2.12	2.60	2.08	2.62	2.99	2.02	0.37	1.88
VCe (%)	9.90	6.43	8.86	6.42	10.25	8.09	9.26	8.12

VCig%: Individual genetic variation coefficient; VCpg%: Progeny genetic variation coefficient; VCe: Error variation coefficient.

Photochemical characters

The overall mean phenotypic values observed for the investigated photochemical traits are depicted in Fig. 9, comprising Photosystem II photochemical efficiency values (F_V/F_M), electron transport per reaction center (ET_0/RC), antenna chlorophyll level specific energy dissipation flux (DI_0/RC), dissipation energy efficiency ($PhiD_0$) and energy conservation performance index from Photosystem II-absorbed photons (Pi_{ABS}) for each season of the year.

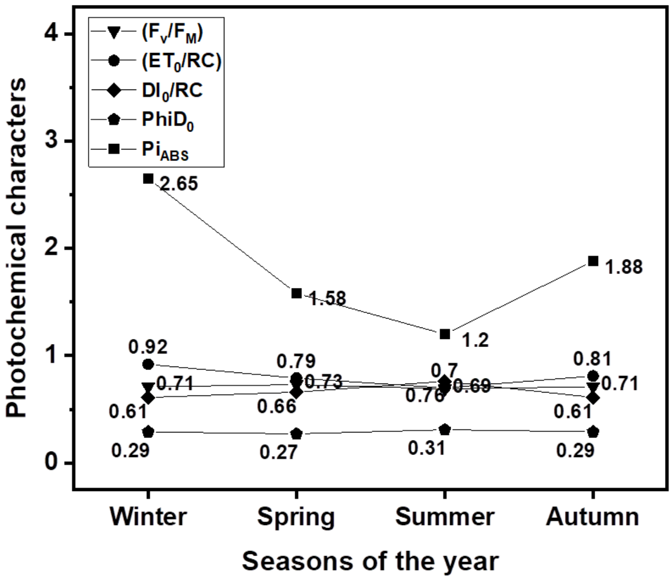

Fig. 9 General photochemical trait averages for each season. (Rio Verde-GO, 2019-2020).

Chlorophyll *a* fluorescence parameters are used to assess the plant photosynthetic apparatuses integrity. However, values observed for these parameters, comprising photochemical characters, represent the general average and, thus, do not faithfully portray field situations. For example, the mean F_V/F_M values remained close to 0.7, which indicates the absence of photoinhibitory damage to the photosynthetic apparatus. However, as this value is obtained from the average, we cannot disregard lower values, indicating higher susceptibility of certain progenies to photoinhibitory effects.

The estimated variance components for the investigated photochemical traits are displayed in fig. 10.

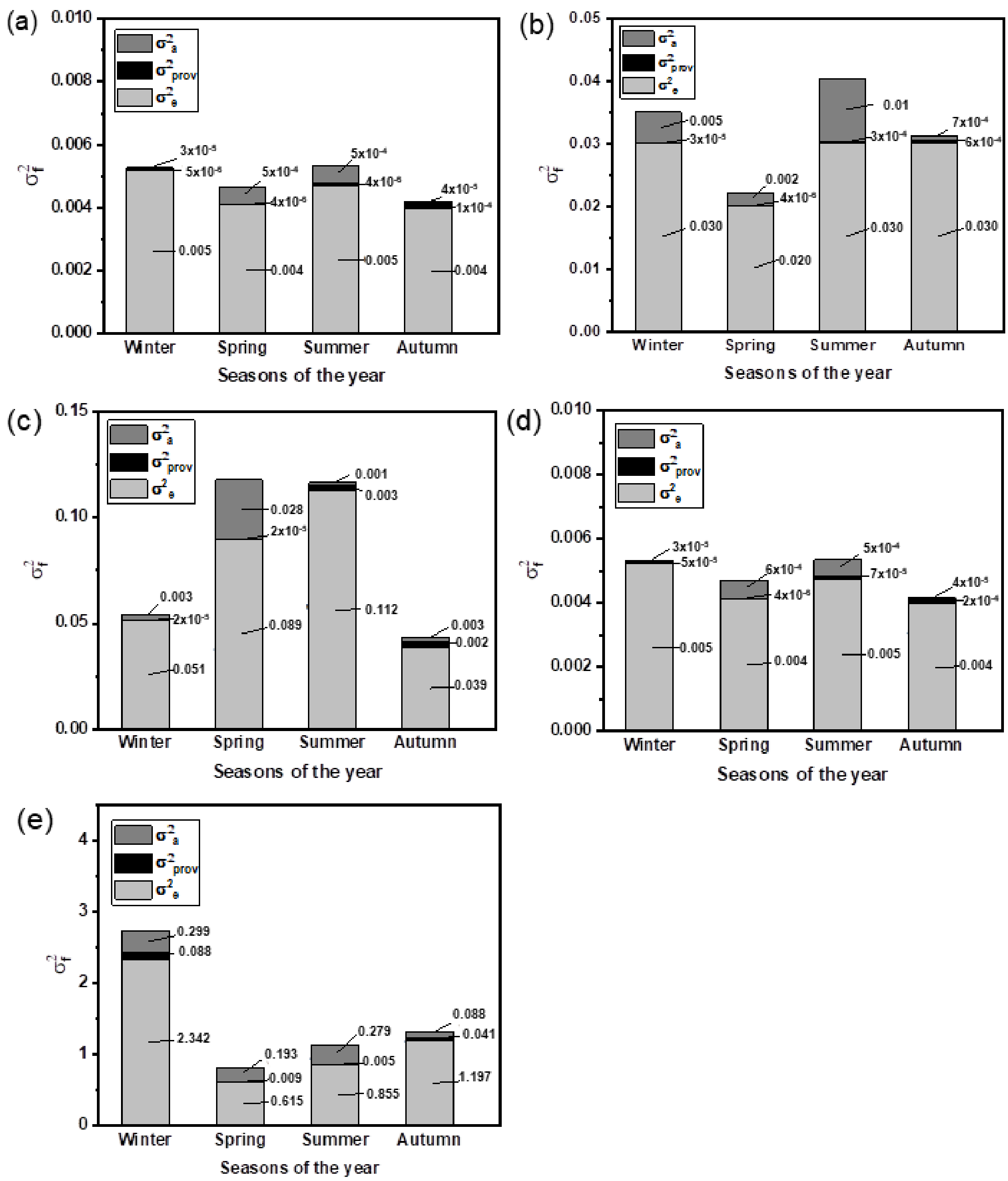

Fig. 10 Variance components for **a** Photosystem II photochemical efficiency (F_V/F_M), **b** electron transport per reaction center (ET_0/RC), **c** antenna chlorophyll level specific energy dissipation flux (DI_0/RC), **d** dissipation energy yield ($PhiD_0$) and **e** energy conservation performance index from Photosystem II-absorbed photons (Pi_{ABS}). σ_f^2: phenotypic variance; σ_a^2: additive variance; σ_{prov}^2: provenance variance; and σ_e^2: error variance.

The σ_a^2 makes up a very small part of σ_f^2 (Fig. 10) for all investigated traits in all seasons, indicating a greater environmental influence. The investigated photochemical traits are directly associated with part of the photosynthesis process. In this regard, the observed environmental influence is due to the ability of energetic environment conditions to affect all biophysical and biochemical processes which, in turn, affect plant metabolism, such as water absorption, respiration, photosynthesis and water loss (Silva et al., 2008).

The h_a^2 estimates for the investigated photochemical traits are depicted in Fig. 11.

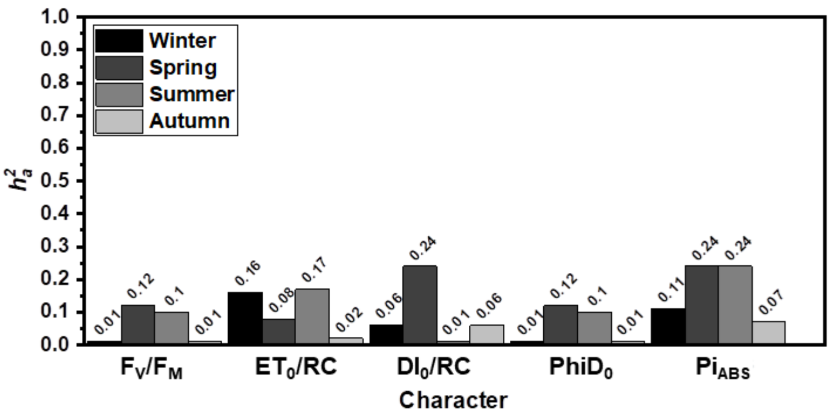

Fig. 11 Narrow sense heritability (h_a^2) concerning the photochemical efficiency of the Photosystem II (F_V/F_M), electron transport per reaction center (ET_0/RC), antenna chlorophyll level specific energy dissipation flux (DI_0/RC), dissipation energy yield ($PhiD_0$) and energy conservation performance index from Photosystem II-absorbed photons (Pi_{ABS}).

Higher h_a^2 estimates were observed for Pi_{ABS} in the summer and spring, and for DI_0/RC in the spring, of 0.24 for both. These traits, along with ET_0/RC in the winter and summer, were categorized as moderate. Lower estimates, in turn, were observed for $PhiD_0$ in the winter (0.01) and autumn (0.01) and Di_0/RC in the summer (0.01).

Despite variations, most traits were categorized as low, although F_V/F_M and $PhiD_0$ presented identical h_a^2 estimates for all seasons. F_V/F_M represents the maximum PSII quantum yield and is directly associated to how much light absorbed by the PSII is used to reduce the Quinone A pool and induce the photochemical process, whereas $PhiD_0$ corresponds to the dissipation energy yield, in other words, non-photochemical dissipation. Except for these traits, which presented equal values, no similar behavior was observed throughout the seasons among the other investigated traits.

Even though photochemical characters may indicate potentially stressful conditions due to environmental causes, these traits were differentially expressed in the present study, with no similarity to chlorophyll index. Despite chlorophyll a being the primary photosynthesis pigment and chlorophyll b an accessory pigment, differential environmental influences were observed regarding index expression compared to chlorophyll a fluorescence trait.

Heritability plays an important role in deciding the adequacy trait selection strategies (Immanuel et al. 2011). In the narrow sense, this parameter quantifies the relative importance of the additive proportion of genetic variance that can be transmitted to the next generation. Lower heritability estimates indicate low genetic trait control, and consequently, low possibilities of obtaining genetic gains for specific traits by mass selection (Zaruma et al. 2015). The increased heritability of a certain trait can be obtained by incorporating higher genetic variability into the population or by minimizing environmental influences (Cruz 2005). In this regard, h_a^2 comprises a property not only of a certain trait, but also of the entire population and specific environmental circumstances (Silva et al. 2018).

The heritable characters identification may indicate the most adequate germplasm use strategies. The differences observed herein concerning physiological trait genetic parameter estimates may have been accentuated by plant age, *i.e.*, initial growth stages, as well as climatic seasonality. To clarify this, the same traits should be continuously determined over time (Codesido and Fernández-López 2008), as barueiro trees are perennial.

The coefficient of variation estimates for the investigated photochemical characters are presented in Table 3.

Table 3 Coefficients of variation for Photosystem II (photochemical efficiency F_V/F_M), electron transport per reaction center (ET_0/RC), antenna chlorophyll level specific energy dissipation flux (DI_0/RC), dissipation energy efficiency ($PhiD_0$) and energy conservation performance index from Photosystem II-absorbed photons (Pi_{ABS})

Parameters	Characters							
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
	F_V/F_M				ET_0/RC			
VCig (%)	0.72	3.27	3.38	0.87	7.64	5.75	11.78	3.27
VCpg (%)	0.36	1.63	1.69	0.43	3.82	2.88	5.89	1.64
VCe (%)	10.23	9.24	10.42	8.87	18.54	19.62	27.45	20.75
	DI_0/RC				$PhiD_0$			
VCig (%)	8.98	25.24	4.90	8.28	1.75	8.90	7.47	2.16
VCpg (%)	4.49	12.62	2.45	4.14	0.88	4.45	3.73	1.08
VCe (%)	37.94	50.04	44.13	33.04	24.97	25.17	23.05	22.04
	Pi_{ABS}							
VCig (%)	20.65	27.74	44.17	15.80				
VCpg (%)	10.33	13.87	22.08	7.90				
VCe (%)	60.55	55.09	86.33	59.94				

VCig%: Individual genetic variation coefficient; VCpg%: Pro genetic coefficient of variation; VCe: Error variation coefficient.

The VCpg for all studied traits were lower than the VCig, indicating that most of the observed variations occur within and not between the investigated progenies. Similar results were reported by Cruz et al. (2020) for *Tachigali vulgaris* plants and by Menegatti et al. (2016) for *Mimosa scabrella*. Thus, it can be inferred that individuals display higher genetic variability at the individual level, denoting the possibility of selecting individuals within progenies.

Under a practical point of view, progeny, and provenance tests for barueiros or other native Brazilian species aiming at greater genetic variability understanding and, consequently, greater genetic gains, should be carried out by sampling a higher progenies number by provenance than the provenances number to be studied.

3.4 Conclusion

This study provides early stage barueiro progeny growth and performance information from an *ex-situ* germplasm bank. Photochemical characters proved to be highly influenced by the environment compared to morphological traits, presenting low magnitude and high heritability estimate variations throughout the year. However, even though the investigated photochemical traits comprise environmental stress responses, differential expressions were noted amongst photochemical and chlorophyll content traits. The greater genetic variability at the individual level observed for the studied traits, on the other hand, indicates the possibility of satisfactory genetic gains applying selection between progenies as a criterion.

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CAPÍTULO II

(**Normas de acordo com a Revista *New Forests***)

Genetic diversity in *Dipteryx alata* Vogel based on morphological and physiological traits in different seasons

Abstract

The *Dipteryx alata* Vogel is a native tree of the Brazilian Savanna which is experiencing extractive exploitation of its fruits. A better understanding of its genetic diversity contributes to its conservation and improvement for future breeding programs. Considering the economic and environmental importance of this tree, our study aims to estimate its genetic diversity using morphological and physiological information for each season of the year, through progeny and provenance tests. For this, 31 half-sib progenies were analyzed and the genetic diversity was measured using the generalized Mahalanobis distance and subsequently the UPGMA (Unweighted Pair Group Method with Arithmetic mean) clustering method, for each season of the year. In addition, it was verified which traits contributed the most to dissimilarity across the seasons, using the Singh's method. The formation of four, three, six and five clusters could be observed in the winter, spring, summer, and autumn, respectively. The estimated cophenetic correlation coefficients were 0.78, 0.80, 0.67 and 0.60 during the winter, spring, summer, and autumn, respectively. The traits that contributed the most to the separation of the progenies into clusters were the chlorophyll contents for all seasons. From the morphological point of view, the height of plants was the most important trait. The results of this study may be used in future management and conservation strategies for this species, and as a model for other species.

Keywords: Barueiro · Generalized Mahalanobis distance · Progeny and Provenance test · Singh · UPGMA Method · Photosystem II

4.1 Introduction

The baru tree (barueiro) (*Dipteryx alata* Vogel) is a native fruit tree from the Brazilian savanna, which has great importance for local populations. This plant has multiple uses: the wood is used for construction of posts, its nut for in natural or processed food, and it also brings advantages for agriculture-livestock integration systems, providing shade and fruits for animals during the dry season (Silva et al. 2021).

Considering its importance, it is necessary to carry out studies that allow its cultivation in a sustainable way, since its exploitation occurs in an extractive way. Studies aiming at its conservation and an intelligent use of its genetic diversity are essential to avoid its extinction, as extractive practices are harmful to the maintenance of its genetic variability (Amélio et al. 2021).

Studies on the influences of the environment and genetics on the expression of its traits are required for the improvement of plant species. Progeny and provenance tests provide this information, as their purpose is to select plants based on their genetic value (Kampa et al. 2020). Furthermore, they serve several important purposes for the conservation of species: preservation of the genetic diversity of a natural population; genetic characterization of the species; and seedling (Avelar et al. 2021).

Morphological traits are usually used to estimate the diversity of populations using phenotypic evaluations (Valadares et al. 2022). However, productivity is directly related to the ability to carry out photosynthesis, and consequently to the photochemical process (Lima et al. 2019). Therefore, physiological parameters can also be used in diversity studies, since they behave as markers of the physiological status of plants.

Obtaining reliable diversity estimates along with genetic parameters are essential to elucidate the genetic structure of populations (Silva et al. 2023). Knowing the degree of genetic variability helps to identify new sources of genes of interest and to indicate genetically distant progenitors for crosses, aiming at the heterotic effect in the hybrid generation.

The multivariate analysis technique has been used to characterize germplasm banks for genetic diversity studies (Ihsan et al. 2021). The method to be used depends on the data set of the analysis to be performed and the required accuracy (Cruz 2013). Among the predictive methods, clustering methods that employ measures of dissimilarity between accessions/progenies are very important (Barth et al. 2022).

Considering the importance of learning more about this species for its conservation, the objective of this study was to measure the genetic diversity among the baru tree progenies, using morphological and physiological information regarding each season of the year, in order to determine which traits contribute the most to the differentiation of progenies/individuals in each season.

4.2 Material and Methods

Genetic material and Seedling Preparation

The genetic material of the *ex-situ* germplasm bank consists of a total of 31 progenies from seeds collected in mother trees from different origins in the State of Goiás (Table 1).

Table 1 Information on the progeny provenance of the baru *ex-situ* Germplasm Bank from the Federal Goiano Institute – Rio Verde Campus

Progeny identification number	Municipality of provenance	Geographic coordinates		Altitude (m)
1	Bom Jardim de Goiás	S 16° 15' 993"	W 052° 02' 186"	362
2	Bom Jardim de Goiás	S 16° 15' 902"	W 052° 03' 138"	407
3	Bom Jardim de Goiás	S 16° 16' 572"	W 052° 03' 704"	389
4	Bom Jardim de Goiás	S 16° 16' 645"	W 052° 03' 552"	377
5	Bom Jardim de Goiás	S 16° 16' 833"	W 052° 02' 621"	413
6	Bom Jardim de Goiás	S 16° 16' 491"	W 052° 02' 927"	403
7	Bom Jardim de Goiás	S 16° 16' 484"	W 052° 02' 027"	402
8	Bom Jardim de Goiás	S 16° 16' 013"	W 052° 02' 878"	387
9	Bom Jardim de Goiás	S 16° 16' 036"	W 052° 03' 198"	389
10	Bom Jardim de Goiás	S 16° 17' 436"	W 052° 01' 174"	389
11	Bom Jardim de Goiás	S 16° 16' 781"	W 052° 03' 584"	392
12	Iporá	S 16° 29' 608"	W 051° 01' 246"	478
13	Iporá	S 16° 28' 138"	W 050° 59' 832"	520
14	Iporá	S 16° 28' 164"	W 050° 59' 853"	516
15	Iporá	S 16° 27' 882"	W 050° 59' 963"	470
16	Iporá	S 16° 28' 069"	W 051° 00' 058"	463
17	Iporá	S 16° 25' 557"	W 051° 09' 369"	514
18	Iporá	S 16° 26' 622"	W 051° 01' 061"	489
19	Urutaí	S 17° 48' 358"	W 050° 54' 374"	713

20	Urutaí	S 17° 15' 235"	W 048° 37' 104"	795
21	Urutaí	S 17° 18' 022"	W 48° 30' 829”	736
22	Urutaí	S 17° 15' 235"	W 048° 37' 101"	731
23	Urutaí	S 17° 29' 238"	W 048° 12' 845"	742
24	Urutaí	S 17° 29' 144"	W 048° 12' 883"	736
25	Ipameri	S 17° 40' 931"	W 048° 09' 506"	838
26	Ipameri	S 17° 39' 646"	W 048° 08' 710"	826
27	Ipameri	S 17° 39' 592"	W 048° 08' 641"	812
28	Ipameri	S 17° 39' 603"	W 048° 08' 759"	823
29	Ipameri	S 17° 39' 347"	W 048° 08' 657"	801
30	Ipameri	S 17° 39' 150"	W 048° 08' 672"	780
31	Ipameri	S 17° 39' 075"	W 048° 08' 619"	762

For each provenance, the matrices were georeferenced and previously chosen with a minimum distance of 100 m between them to avoid collecting fruits on trees with a high degree of kinship, thus maximizing genetic variability.

After the collection, the fruits were properly packaged to avoid mechanical damage and then transported to the Plant Tissue Culture Laboratory, at the Federal Goiano Institute, Rio Verde campus; where they were stored until the seeds were removed using coconut breaker equipment. Sowing was carried out in tubes with a capacity of 290 cm3 of substrate, which were composed of: ravine soil, carbonized rice husks and vermiculite in a 1:1:1 ratio. The seedlings were produced in the open air and taken to the field 120 days after sowing. Irrigations were carried out three times a day.

Area and Study Design

The *ex-situ* germplasm bank was established on February 23, 2018 in an experimental area at the Federal Goiano Institute, - Rio Verde campus (17°48'36" S, 50°54'10" W). The planting area was previously harrowed and furrowed with the aid of a furrower at the desired spacing between rows. The design used was randomized blocks, with 10 repetitions, one plant per plot, spaced 5 x 4m, totaling 310 plants. A fertilizer was used in the area according to the soil analysis results (Table 2).

Table 2 Soil analysis of the baru *ex-situ* Germplasm Bank area at Federal Goiano Institute – Rio Verde Campus. Rio Verde – GO – 2017

Depth	Ca	Mg	Al	H+Al	CTC	K		S	P	M.O
				$cmol_c\ dm^{-3}$				$mg\ dm^{-3}$		
0-20 cm	2.7	0.9	0.05	4.2	8.1	0.26	103	4.8	3.3	31.4
20-40 cm	2.5	0.8	0.10	3.3	6.8	0.19	75	5.0	1.0	23.7
	Clay	**Silt**	**Sand**	**pH**	**SB**	**Base Saturation**				
		%		**$CaCl_2$**	**V%**	**M%**				
0-20 cm	58	10	32	4.90	48	1.3				
20-40 cm	61	10	29	4.61	52	2.8				

Traits Assessed

The analyses started in July 2019, when the plants were 16 months old, and ended in June 2020. The plants were evaluated for morphological and physiological traits.

Morphological Approach

The morphological traits evaluated were: plant height and stem diameter. Plant height was measured using a graduated ruler, from the ground to the insertion of the last leaf, and the stem diameter was measured using a digital caliper, approximately three centimeters above the ground. Five evaluations were made in each season of the year for these traits.

Physiological Approach

The physiological traits evaluated were: chlorophyll values (chlorophyll *a*, chlorophyll *b* and chlorophyll total) and chlorophyll *a* fluorescence (Photosystem II photochemical efficiency - F_V/F_M, electron transport per reaction center - ET_0/RC, antenna chlorophyll level specific energy dissipation flux - DI_0/RC, dissipation energy efficiency - $PhiD_0$ and energy conservation performance index from Photosystem II-absorbed photons - $Pi_{ABS)}$. Chlorophyll values were evaluated five times in each season using an electronic chlorophyll meter. - ClorofiLOG (model CFL 1030, Falker Automação Agrícola, Brasil) and the chlorophyll *a* fluorescence parameters were measured using a fluorometer, FluorPen, model F100 (*Photon systems Instruments*). Measurements were performed on completely expanded healthy and dark-adapted leaves for 30 minutes with the aid of tweezers. For photochemical traits, the observations consisted of only one evaluation for all individuals at the end of each season, due to logistic reasons.

Data were obtained for all traits in all seasons of the year.

A multivariate analysis was carried out using the Mahalanobis distance to verify the dissimilarity of the progenies and, later, using the UPGMA clustering method, the progenies were clustered in a way that the similar ones occupied the same cluster, thus forming the dendrogram.

The importance of traits for dissimilarity was measured using the Singh's method (1981).

The *softwares* Genes (Cruz 2016) and Rbio (Bhering 2017) were used.

4.3 Results and discussion

The cluster analysis using the UPGMA method based on the Mahalanobis matrix separated the 31 progenies into clusters of individuals with genetic proximity (similarity), showing different allocations of progenies and numbers of clusters across the seasons (Fig. 1).

Four clusters were formed in winter, three in spring, six in summer and five in autumn. The clusters formed in winter (Fig. 1a) were composed of the following progenies: 18 (cluster 1); 7 (cluster 2); 25 and 30 (cluster 3); and 17, 29, 19, 28, 24, 16, 26, 15, 27, 6, 10, 31, 13, 21, 9, 11, 5, 14, 20, 3, 22, 12, 23, 2, 8, 1 and 4 (cluster 4). The clusters formed in the spring (Fig. 1b) were composed of the progenies: 14 (cluster 1);10 (cluster 2); and 3, 24, 7, 26, 17, 29, 22,20, 11, 2, 4, 9, 12, 23, 1, 8, 13, 19, 18, 31, 5, 15, 25, 6, 27, 30, 28, 16 and 21 (cluster 3). The clusters formed in the summer (Fig. 1c) were composed of the progenies: 7 (cluster 1); 21 (cluster 2); 30 (cluster 3); 19 (cluster 4); 12 and 22 (and 5); and 5, 2, 14, 4, 1, 8,15, 27,11,16, 17, 29, 24, 26, 6, 18, 28, 3, 20, 9, 23, 13, 25, 10 and 31 (cluster 6). The clusters formed in the Autumn (Fig. 1d) were composed of the progenies: cluster 1 (12), cluster 2 (30), cluster 3 (29, 31, 10, 18, 22, 23, 17, 27, 25, 15, 21, 13, 16 and 28), cluster 4 (5 and 6) and cluster 5 (3, 19, 24, 9, 2, 26, 4, 20, 1, 7, 8, 11 and 14).

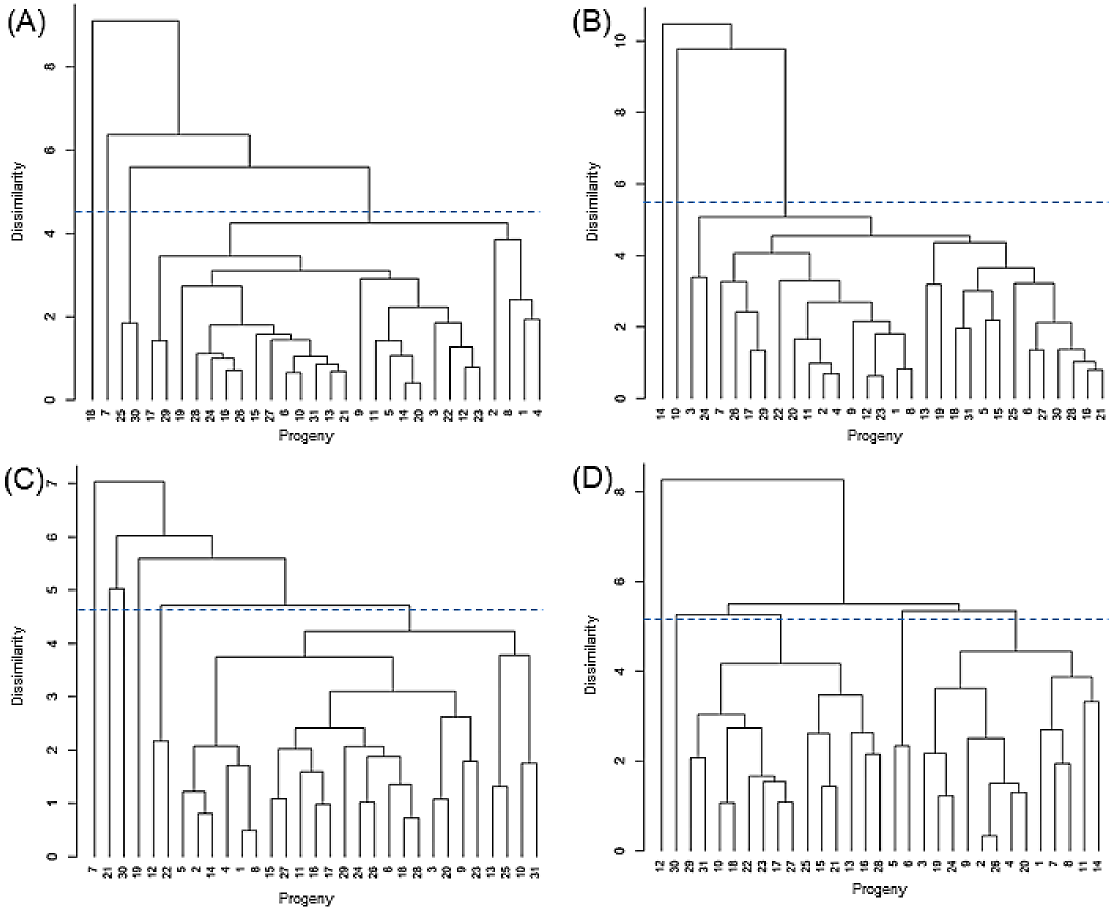

Fig. 1 UPGMA dendrogram obtained through Mahalanobis distance for seasons **a** winter (CCC: 0.78), **b** spring (CCC: 0.80), **c** summer (CCC: 0.67) and **d** autumn (CCC: 0.67): 0.60).

These results show that progenies from different provenances were allocated in the same cluster; therefore, progenies from the same provenances were allocated in different clusters. Thus, the occurrence of diversity between progenies from different provenances and among individuals from the same location can be observed, which demonstrates greater variability within populations, not between them. Furthermore, in each season the configuration of the clusters changed completely, which could be a consequence of environmental influences on traits.

The results observed in this study indicate that large part of the genetic variation of the species is within a population (intrapopulation genetic diversity). Similar results were found by Lima et al. (2020), in natural populations of *Astrocaryum acaule* Mart, demonstrating that this same trend seems to occur in *in-situ* conservation and in different species. This is due to reproductive and demographic patterns, as species with high crossing rates and high population density present less divergence between populations than those with higher self-fertilization rates and low population density (Kageyama et al. 2003).

Supporting the results observed in this study, using RAPD markers in baru populations in São Paulo, Minas Gerais and Goiás, Soares et al. (2008), it was found that 12% of the total variation is between populations, while 88% of the total variation is within the population (intrapopulation diversity). Likewise, it was observed by Silva et al. (2023) a high diversity (90%) within native populations of baru trees in the Brazilian savanna, using microsatellite markers.

This measured diversity provides a baseline for studies involving selection of materials with desirable traits. In addition, it paves the way for further studies on the potential of this fruit farming business. Based on the observed trend, selection strategies or even further studies can be planned, since the selection will not be based on either cluster presented herein, but they might help to detect the possibility of early selection, when correlated to productive traits of interest.

The proper representation of the dendrogram in relation to the real data, the cophenetic correlation estimate, showed different coefficients, ranging from 0.60 to 0.80 for the autumn and spring seasons, respectively. According to the criterion proposed by Rohlf (1970), values lower than 0.70 indicate that the clustering process is inadequate to summarize the data set information, however, some authors consider that in certain situations this criterion becomes outdated (Bezerra Neto et al. 2010).

The study of genetic diversity, obtaining information about the formation of clusters in a given population is important for choosing possible parents, since the new combinations to be established must be based on the degree of their dissimilarities and on the potential of the parents (Cruz et al. 2011). Therefore, the crossing of individuals that present the same pattern of dissimilarity may lead to unsatisfactory gains through selection. In addition to dissimilarities, it is important that parents associate high average and variability of the traits to be improved (Melo et al. 2019).

The phenotypic averages for all evaluated traits are presented by clusters created in the winter clustering season (Fig. 2). For morphological traits, cluster 3 had the highest average, while cluster 2 had the lowest average (Fig. 2a) among the four clusters formed (Fig. 1a). Phenotypic values for chlorophyll index showed close mean values between clusters (Fig. 2a). As for the photochemical traits, Pi_{ABS} was the one that changed the most the average of the clusters, with the highest value (4.53) in cluster 1 and the lowest value in cluster 3 (1.9).

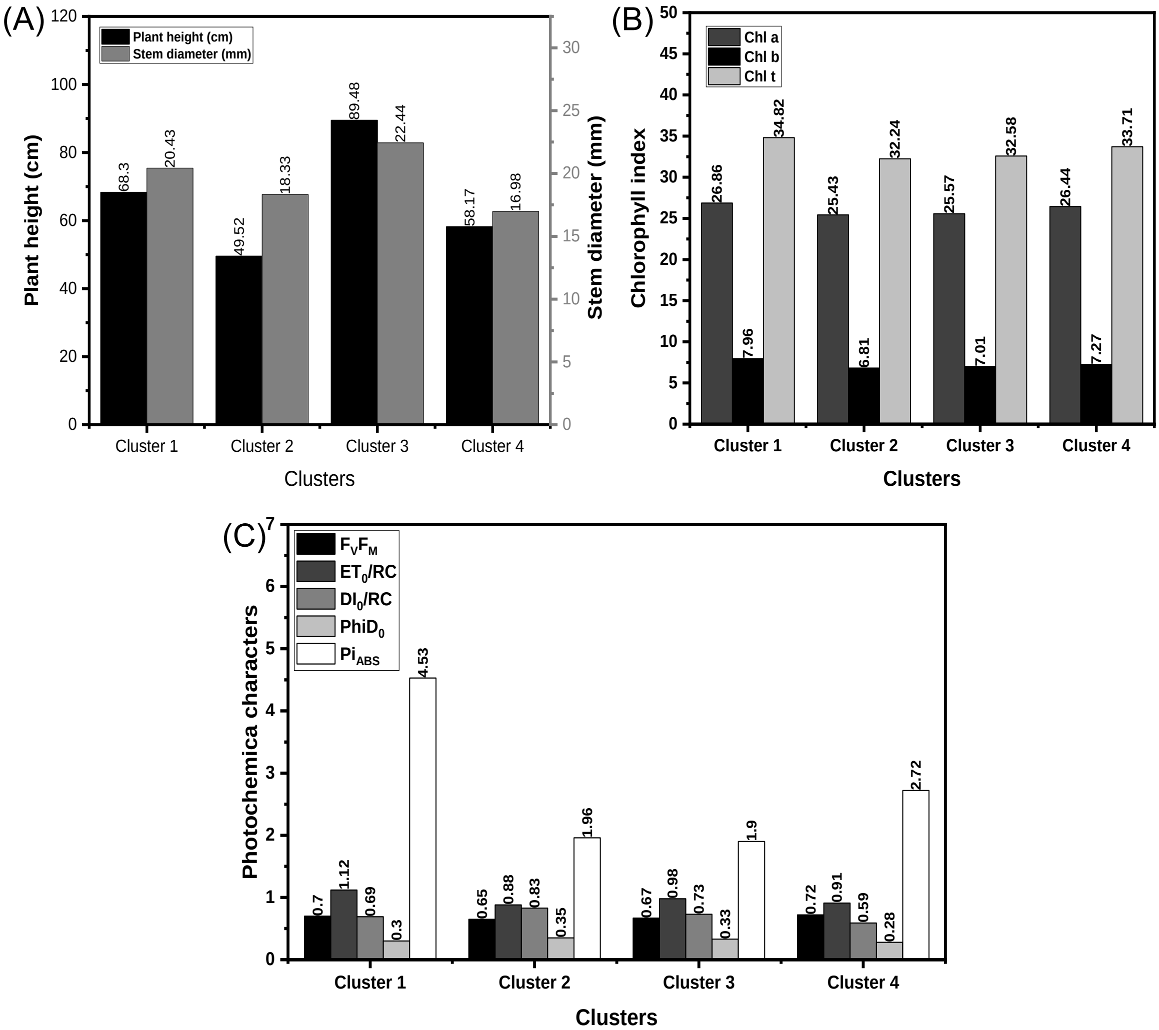

Fig. 2 Average phenotypic values for traits **a** morphological, **b** chlorophyll indices and **c** photochemical of the progenies of clusters formed in the winter season.

The phenotypic averages for all evaluated traits are presented by clusters formed in the spring season clustering (Fig. 3). The stem diameter trait showed close values (19.25 - 21.33 mm) for the average of the clusters, while for the plant height trait the clusters showed greater differentiation, with 78.12 cm for cluster 1 and 97.93 cm for cluster 2 (Fig. 3a). As for chlorophyll index, cluster 2 was outstanding in all traits, with 2.86, 8.02 and 36.74 for chlorophyll *a*, *b* and total, respectively (Fig. 3b). Of the photochemical traits, ET_0/RC, Di_0/RC and Pi_{ABS} were the ones that showed the greatest differences between the formed clusters (Fig 3c).

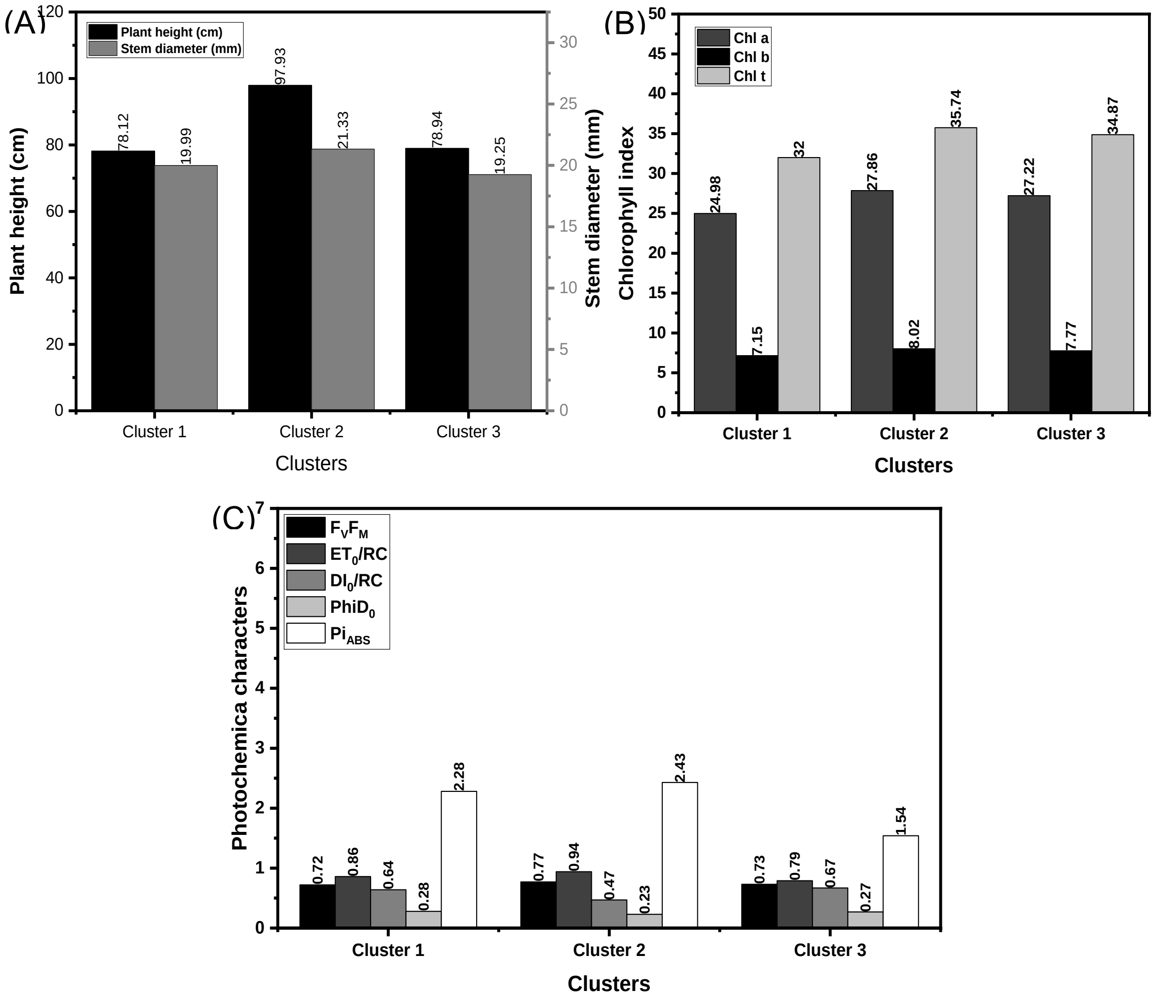

Fig. 3 Average phenotypic values for traits **a** morphological, **b** chlorophyll indices and **c** photochemical of the progenies of clusters formed in the spring season.

The phenotypic averages for all evaluated traits are presented by clusters formed in the summer season clustering (Fig. 4). The average values for plant height in the formed clusters ranged from 72.99 (cluster 5) until 144.29 cm (cluster 4), while the stem diameter also showed lower and higher values for the same clusters, being 19.33 and 35.56 mm, respectively (Fig. 4a). Phenotypic values for chlorophyll values showed close mean values between clusters (Fig. 4b). For photochemical traits, Pi_{ABS} showed great differentiation between clusters, with emphasis on cluster 3 (2.05) with the highest mean value, while cluster 1 had the lowest mean value with 0.57 (Fig. 4c).

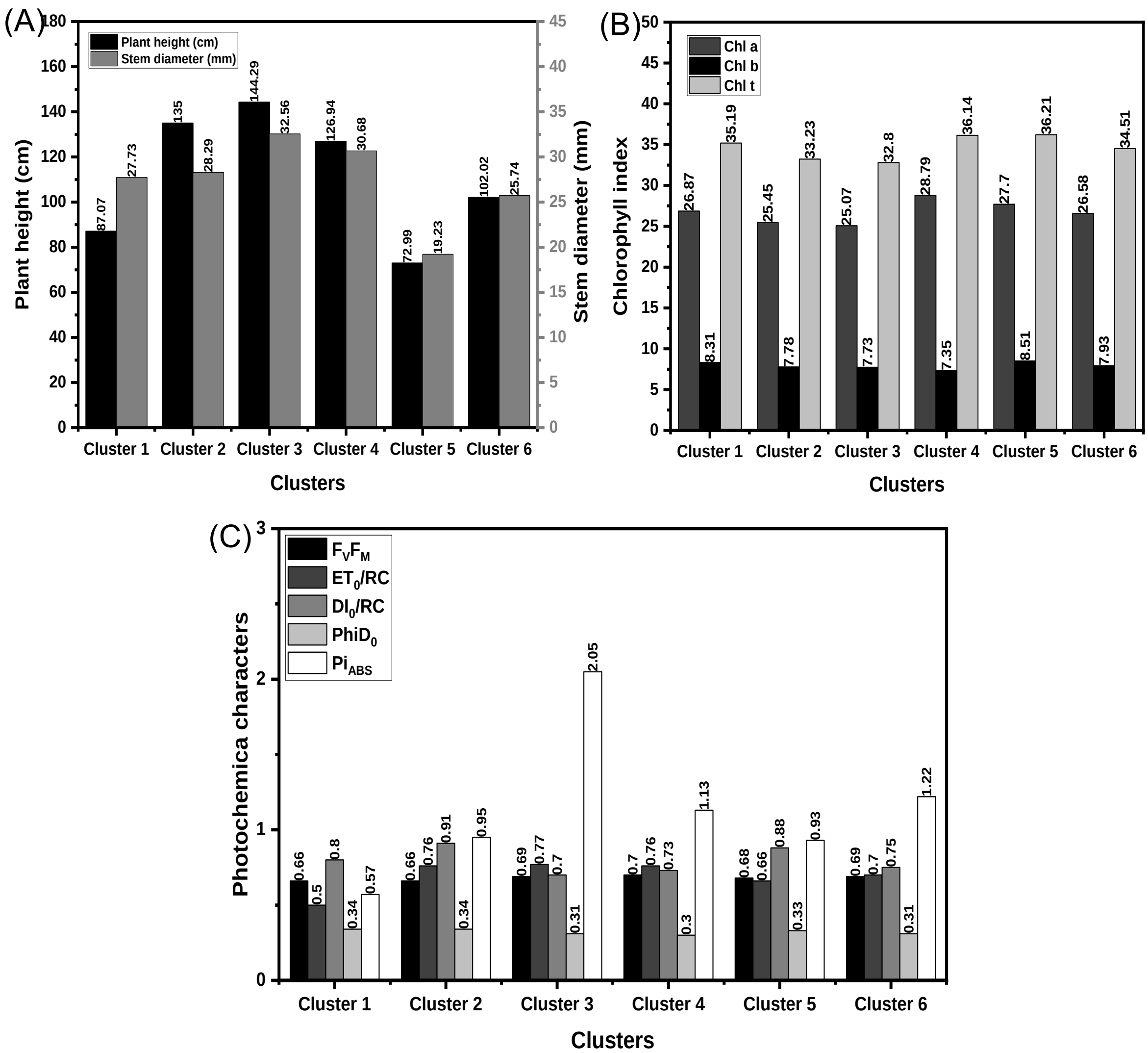

Fig. 4 Average phenotypic values for traits **a** morphological, **b** chlorophyll indices and **c** photochemical of the progenies of clusters formed in the summer season.

The phenotypic averages for all evaluated traits are presented by clusters formed in the autumn season clustering (Fig. 5). For morphological traits, cluster 2 had the highest averages, while cluster 1 had the lowest averages among the four clusters formed (Fig. 5a). As in the formation of clusters in the other seasons, the phenotypic values for chlorophyll values showed close average values between clusters (Fig. 5a) and the greatest differentiation of photochemical traits occurred with Pi_{ABS} (Fig.5a), ranging from 1.32 (cluster 1) until 3.11 (cluster 4).

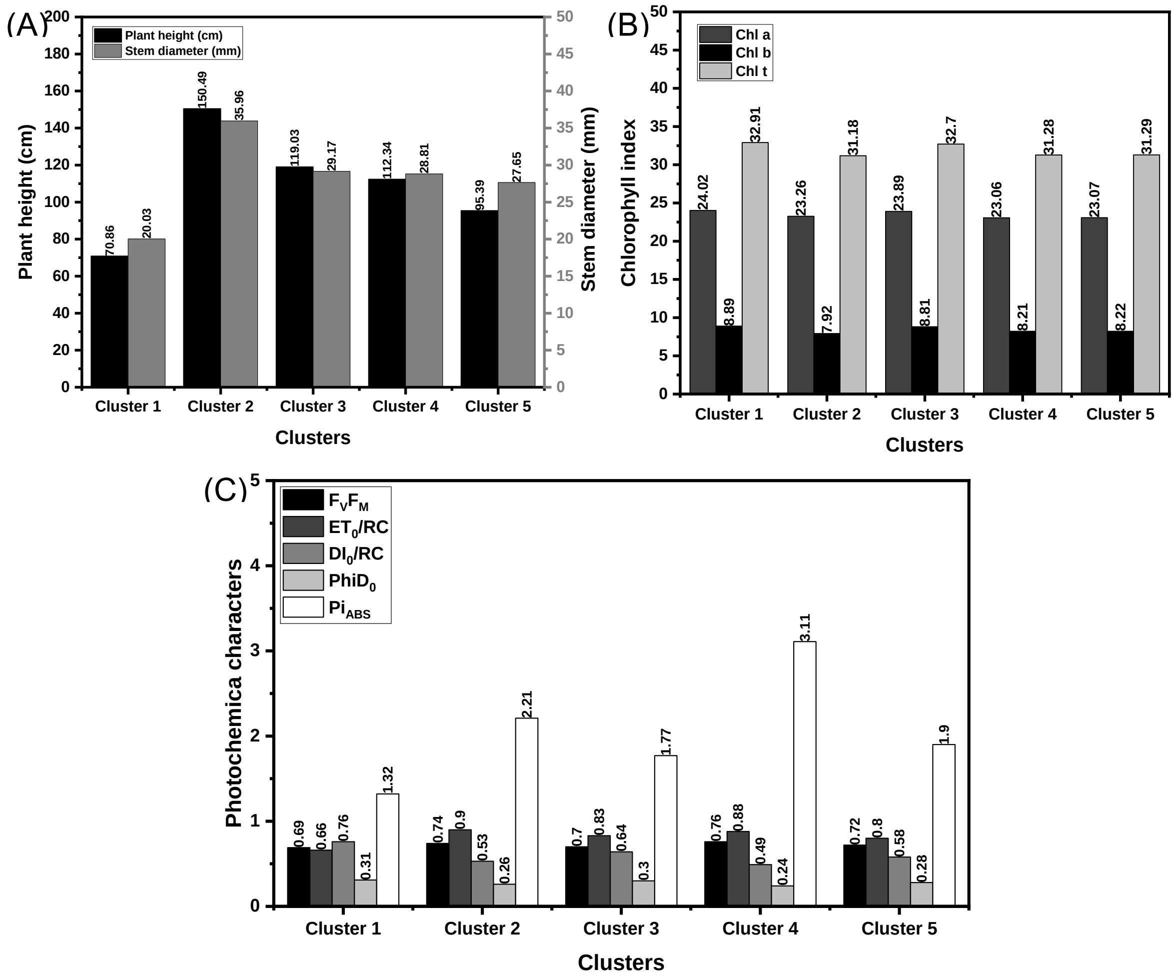

Fig. 5 Average phenotypic values for traits **a** morphological, **b** chlorophyll indices and **c** photochemical of the progenies of clusters formed in the autumn season.

The relative contributions to dissimilarity of all traits are shown in Fig. 6. In all seasons, it was observed that some of the chlorophyll values were the traits that contributed the most. In the winter season, it was chlorophyll b, in spring, total chlorophyll, and in summer and autumn, chlorophyll a contributed the most to diversity.

As for morphological traits, plant height contributed the most in all seasons, with values of 14.86% (winter), 23.26% (spring), 4.79% (summer) and 16.45% (autumn), while stem diameter showed practically zero value. The photochemical traits showed a greater contribution in the winter (Fig. 6a) and autumn (Fig. 6d) seasons. Among the photo chemicals, the one that contributed the most was Pi_{ABS} followed by Di_0/RC.

According to the Singh's criterion (1981), it was observed that in the winter season (Fig.6a) only the trait chlorophyll b contributed to 58.32% of the genetic divergence. In the spring season (Fig. 6b), the traits total chlorophyll and plant height contributed to 69.21%. In the summer season (Fig. 6c), only chlorophyll a contributed to 61.3%, while in the autumn season (Fig. 6d) the traits chlorophyll a and chlorophyll b contributed to 62.88% for genetic divergence.

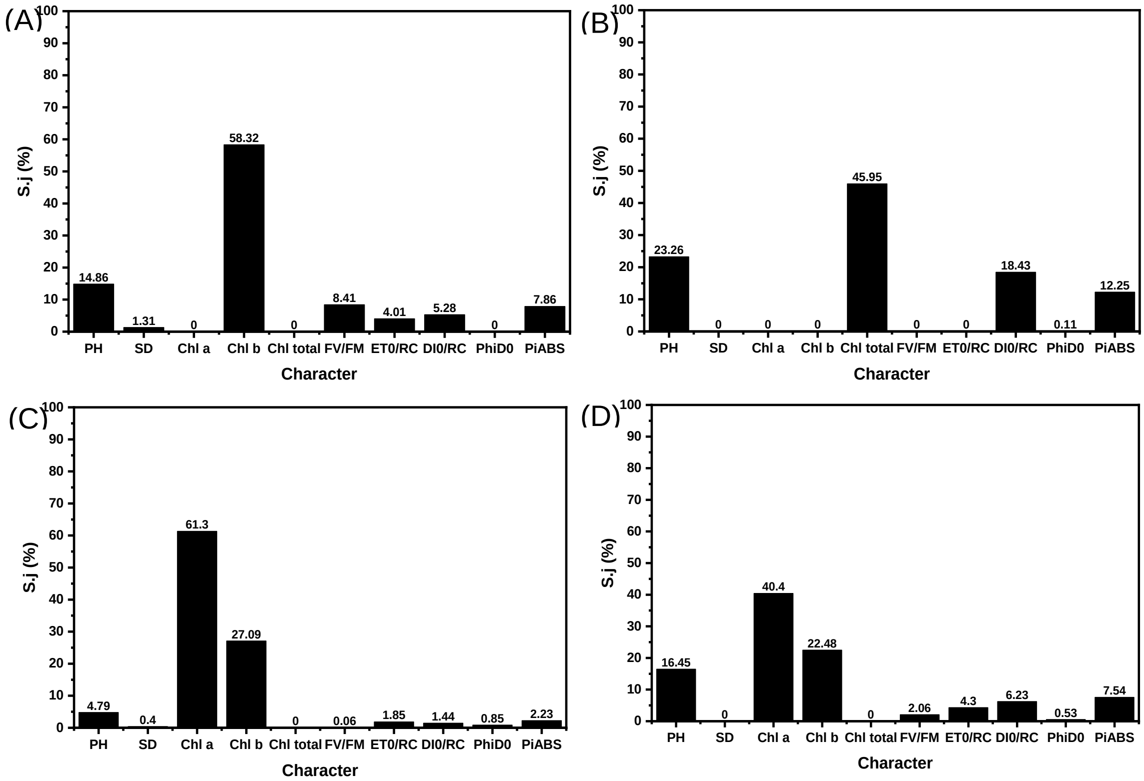

Fig. 6 Relative contribution (S.j) of all traits to genetic dissimilarity in seasons **a** winter, **b** spring, **c** summer and **d** autumn. PH: plant height, SD: stem diameter, Chl a: chlorophyll a index, Chl b: chlorophyll b index, Chl total: total chlorophyll index, FV/FM: F_V/F_M - photosystem II photochemical efficiency, ET0/RC: ET_0/RC - electron transport per reaction center, DI0/RC: DI_0/RC - antenna chlorophyll level specific energy dissipation flux, PhiD0: $PhiD_0$ - dissipation energy efficiency, PiABS: Pi_{ABS} - energy conservation performance index from Photosystem II-absorbed photons.

Through this analysis, it is possible to classify studied traits according to their contribution and eliminate those with lesser contribution. The elimination of traits facilitates future studies on dissimilarity in terms of work and costs, making them more feasible. Therefore, the stem diameter trait could be eliminated, as it did not contribute to any of the stations.

As mentioned before, the chlorophyll index did not show great differences in the average phenotypic values in clusters formed in all seasons (Fig 2-5), being of great importance for the identification of genetically similar individuals (Fig 6). This was due to the influence of the environment on the expression of these traits, causing the genotypic values, which determine the clusters, to behave differently compared to the phenotypic values.

The characterization of the germplasm bank, since its formation through all stages of development, is extremely important for future decisions on the genetic resources contained in this bank. Considering that the baru tree is a perennial species, further studies should be conducted using repeated measurements over time, to learn about the behavior of the progenies, making it possible to obtain the maximum amount of information for use or conservation and future selection of superior genotypes.

4.4 Conclusions

This study demonstrated the existence of genetic variability among the evaluated progenies.

The UPGMA method led to the formation of clusters of different configurations throughout the year, in terms of number of clusters and individuals allocated to them.

The chlorophyll indexes were of major importance for the formation of clusters. In each season of the year, at least one or more index was the highest contribution.

This study presents information that can be used for decision-making in future management and conservation strategies regarding the studied germplasm bank, and it may serve as a model for other species.

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CONCLUSÃO GERAL

Com as estimativas dos parâmetros em barueiros em fase juvenil obtidas a partir da mensuração de caracteres morfológicos e fisiológicos pode-se verificar que os caracteres fisiológicos de natureza fotoquímica apresentaram ser altamente influenciados pelo ambiente, sendo baixa a influência genética na expressão deles. Por outro lado, a altura de plantas foi o caractere morfológico de maior herdabilidade

O método UPGMA demonstrou variabilidade entre as progênies avaliadas formando quatro, três, seis e cinco grupos nas estações inverno, primavera, verão e outono, respectivamente. Os caracteres que mais contribuíram para a separação das progênies em grupos foram os índices de clorofila em todas as estações.

A existência de maior variabilidade genética entre progênies observada para as características estudadas indica a possibilidade de ganhos genéticos satisfatórios tendo como critério a seleção entre progênies, não entre populações de diferentes procedências.

Há variabilidade genética nas plantas de barueiro da coleção de germoplasma do IF Goiano- campus Rio Verde, entretanto, são necessários maiores estudos ao longo do desenvolvimento das plantas para geração de maiores informações a fim de favorecer a conservação e seleção futura de materiais de interesse comercial.