



FORESTRY SCIENCE

Phenotypic divergence among half-sib progenies of African mahogany

AGNES C. CRUZ, PRISCILA A. BARROSO, BRUNA A.S. DIAS, SILVOKLEIO C. SILVA & JOAQUIM S. LIMA

Abstract: Divergence studies are essential for the conservation of genetic resources and guidance in genetic improvement programs. This study aimed to assess the phenotypic dispersion of half-sib progenies using Gower's algorithm and the non-metric multidimensional scaling analysis based on qualitative and quantitative traits. Twenty-two half-sib progenies of African mahogany (*Khaya senegalensis*) were evaluated in a randomized block design with 22 treatments (progenies/origin), with 30 replications and one plant per plot, totaling 660 plants. The total tree height (cm), diameter at soil level (cm), chlorophyll A and B, presence of bifurcation, presence of lateral branches, stem tortuosity, and the phytosanitary and nutritional status according to a rating scale were evaluated. Data analysis was performed at the progeny mean level for the quantitative traits and the modal value was used for the qualitative traits. The technique used presented the distance between the progenies of *K. senegalensis* in a graphical form. The dispersion of the progenies in the graph is indicative that there is phenotypic variation in the progeny test, allowing to select individuals with characteristics of interest to improve mahogany for Piauí. Gower's algorithm applied to mixed data and non-metric multidimensional scaling are efficient methods to describe phenotypic similarities between progenies.

Key words: *Khaya senegalensis*, genetic diversity, Gower's distance, multivariate analysis, morphological markers.

INTRODUCTION

The Brazilian forestry sector is considered one of the most developed in the world (FAO 2015). This sector serves as the basis for important industry segments that use wood as raw material (M.S. Schuchovski, unpublished data). The growing demand for wood and the concern to preserve remaining natural areas have boosted the cultivation of forest species for commercial purposes. Thus, the challenge for this sector is to intensify production in order to meet this demand, especially for timbers (IBÁ 2017). African mahogany (*Khaya senegalensis*), belonging to the genus *Khaya*, has gained space

in this type of cultivation, especially due to its added value. This wood is intended for use in the making of furniture, shipbuilding, sophisticated construction, decorative laminates, flooring, and household items, among others (Arnold 2004).

African mahogany is native to Africa and was initially introduced in the northern region of Brazil, in the 1970s, as an alternative to the exploitation and commercialization of Brazilian mahogany, which is considered a hardwood (Silva 2013, Ribeiro et al. 2017), making its marketing unfeasible in addition to its high susceptibility to *Hypsipylla grandella* (mahogany shoot borers). In contrast to Brazilian mahogany, the African mahogany is resistant to mahogany shoot borers

(Maués 2001, Verzignassi et al. 2009, Pinheiro et al. 2011, Tremacoldi et al. 2013, Soares 2014) and has very similar wood characteristics (Pinheiro et al. 2011), which has brought attention to its production in other regions of the country (IBÁ 2017), including the Brazilian northeast.

Internationally, it is estimated that the value of the sawn and dried wood is approximately €\$ 1,000 per m³ (IBF 2020), corresponding to more than 30 times the m³ value of other hardwood species, such as pine and eucalyptus, which are widely used for commercial cultivation (IBGE 2017). However, in order to achieve this high profitability and success in African mahogany forest plantations, seedlings of good genetic and morphophysiological quality are required, as well as superior genetic materials, with adaptive potential to local conditions (Carneiro 1995).

Provenance and progeny trials (TPP) are essential steps for the domestication and use of plant species for commercial cultivation, enabling strategies for selecting and characterizing inheritance patterns in forest species (Rocha et al. 2009). This type of research has shown that most tree species have a high degree of phenotypic variation under different edaphoclimatic conditions (Rehfeldt et al. 2002) and that this varies between provenances (Aitken et al. 2008). Therefore, genetic divergence studies based on phenotypic traits support the selection process in genetic improvement programs and are essential for the conservation of genetic resources.

Studies on plant divergence and selection are mainly supported by multivariate techniques (Cruz et al. 2014), which show connections, differences, or similarities between traits (Hair Jr et al. 2009). The main techniques used in divergence studies of forest species are clustering and principal component analysis (Soares 2014, Azevedo et al. 2015, Domiciano et al. 2015, Giustina et al. 2017). The cluster

analysis is mainly based on the Euclidean and Mahalanobis distances, which consider only the quantitative traits. However, it can be affirmed that qualitative traits are also important to characterize the performance of high-quality seedlings and plants that meet the demands of the market. In the development of the African mahogany, for example, it has been noted that traits such as stem shape and the presence of lateral branching present variability between and within progenies. These and other traits that are categorically evaluated can and should be considered in divergence and selection studies within improvement programs.

The use of the maximum information available within the improvement program, for divergence studies, can be performed through Gower's algorithm (Gower 1971). In this technique, one algorithm estimates the similarity between individuals using data with discrete and continuous distributions, allowing to obtain the distance between individuals based on qualitative, quantitative, and mixed data (Moura et al. 2010).

Complementary, using non-metric multidimensional scaling (nMDS), it is possible to graphically represent the distances between analyzed individuals. The advantage of the nMDS is that this method makes no restrictions on the type of data that originated the distance matrix and can be easily used along with the distances obtained by Gower's algorithm (Silva 2016). The nMDS method has already been used in phenotypic divergence studies with species of agronomic interest, such as cocoa (Leal et al. 2008), garlic (Silva et al. 2014), lima-bean (Barroso et al. 2019) and ornamental pepper (Pessoa et al. 2019). However, no information on the use of this technique for forest species was found for genetic improvement purposes.

There are no recommendations for African mahogany progenies that would be suitable for

specific regions in Brazil, such as the state of Piauí. This information would provide subsidies for future genetic improvement programs in order to achieve a greater potential for the species and generate innovative technologies for the forestry sector. Thus, the present study aimed to evaluate the phenotypic dispersion of half-sib progenies of African mahogany (*Khaya senegalensis*) using Gower's algorithm and non-metric multidimensional scaling, based on qualitative and quantitative traits.

MATERIALS AND METHODS

The experiment was conducted in March 2017, at the Alvorada do Gurguéia School Farm, located in the municipality of Alvorada do Gurguéia, southwest region of the state of Piauí, Brazil.

The genetic materials used were originated from 22 progenies of *Khaya senegalensis* that were selected and properly identified in a region with natural occurrence of the species and superior silvicultural characteristics. The seedlings were grown in tubes at the Futuro Florestal/Tropical Flora company in the state of São Paulo and manually planted in the experimental areas with a 3 m x 3 m spacing, following the standard methodology of the company.

The half-sib progenies of African mahogany (*Khaya senegalensis*) were evaluated in the early phase (20 months after planting). A randomized block design was used in this study, composed of 22 treatments (progenies /provenance), 30 replicates, and one plant per plot, totaling 660 plants.

The evaluated traits were: total tree height (cm) and stem diameter at soil level (cm), both measured with a measuring tape; chlorophyll A and B, measured with a Falker® digital chlorophyll meter; presence of bifurcation, presence of lateral branches, stem tortuosity (tortuous or

rectilinear), phytosanitary status (satisfactory or unsatisfactory), and nutritional status (deficient or not deficient).

Data analysis was performed at the progeny mean level for the quantitative traits, and the modal value was used for the qualitative traits. Three distance matrices were constructed: (i) using only quantitative traits; (ii) using qualitative traits; (iii) using qualitative and quantitative traits together, called mixed data matrix. The distance matrix based on quantitative variables was constructed by using the generalized distance of Mahalanobis (Mahalanobis 1936). A matrix with qualitative data was constructed using Gower's distance (Gower 1971). The joint data analysis of qualitative and quantitative data, here identified as mixed data, was also performed by Gower's method.

Afterward, the three distance matrices were separately represented by non-metric multidimensional scaling (nMDS). The lack of adjustment in each nMDS mapping was calculated using Kruskal's stress, which indicates stress values up to 20% as satisfactory (Kruskal 1964). Furthermore, the table of stress values proposed by Sturrock & Rocha (2000) was used to validate the result obtained with the nMDS solution by comparing the value obtained with the stress value resulted from matrices with the same number of objects (n= 22 progenies) and the same number of dimensions (k= 2 dimensions). All statistical analyses were made using the software R 3.6.0. (R Core Team 2019).

RESULTS

There was no variation among progenies for stem tortuosity and phytosanitary and nutritional characteristics, being considered monomorphic. All progenies were classified as having rectilinear stems, and the phytosanitary and nutritional status were classified as satisfactory.

The remaining morphological, quantitative, and qualitative traits were efficient in discriminating the 22 progenies/provenances of *K. senegalensis* from the UFPI/CPCE (Universidade Federal do Piauí/Campus Professora Cinobelina Elvas) (Figure 1). The dispersion among progenies for the three representations showed phenotypic diversity in the studied population.

The scaling showed a 0.03% stress value for the data, 15.98% for the quantitative data, and 14.09% when using mixed data. The stress value for qualitative data is considered excellent, whereas the stress for quantitative and mixed data is reasonable, according to Kruskal’s classification (Kruskal 1964). When evaluating the critical values ($\alpha= 0.01$) established by Sturrock & Rocha (2000), it is possible to affirm that the progenies are not randomly arranged in the bidimensional plane of the scaling for the three representations, indicating a good ordering.

It can be seen in the qualitative data that the progenies formed three well distinct clusters (Figure 1a) Group 1 was formed by progenies 10, and 17; group 2 by progenies 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 15, 18, 19, 20, and 21; and group 3 by progenies 14, 16, and 22. The progenies of group 1 present bifurcation and the absence of lateral branches as common characteristics. The progenies of group 2 have a rectilinear stem, without bifurcation, and without lateral

branches. The progenies of group 3 present lateral branches and no bifurcation.

Considering the quantitative data (Figure 1b), it is possible to notice a greater dispersion of the progenies, resulting in a greater contribution to divergence. Some clusters are observed in the graphic, as well as divergent progenies. Progeny 3 is more distant in the bidimensional plane and is therefore considered the most divergent. This progeny presents a lower mean (Table I) compared to the remaining progenies for the diameter at soil level (19.34 mm) and height variables (59.25 cm) and is therefore not indicated for genetic improvement. Progeny 3 also showed the highest chlorophyll-a index (Table I), followed by progeny 10, the closest to the first in the bidimensional plane. Progenies 1, 7, 10, and 18 are also isolated but are less divergent from the others. Among these, progeny 1 can be highlighted for presenting one of the more significant developments considering the average plant height (106.29 cm). It is noted that this progeny is very close to the remaining progenies with good height development, such as progenies 5 and 17. In general, the lower quadrant progenies showed lower development in height and diameter at soil level.

In the nMDS for mixed data (Figure 1c), it was possible to better discriminate between studied progenies due to the greater dispersion,

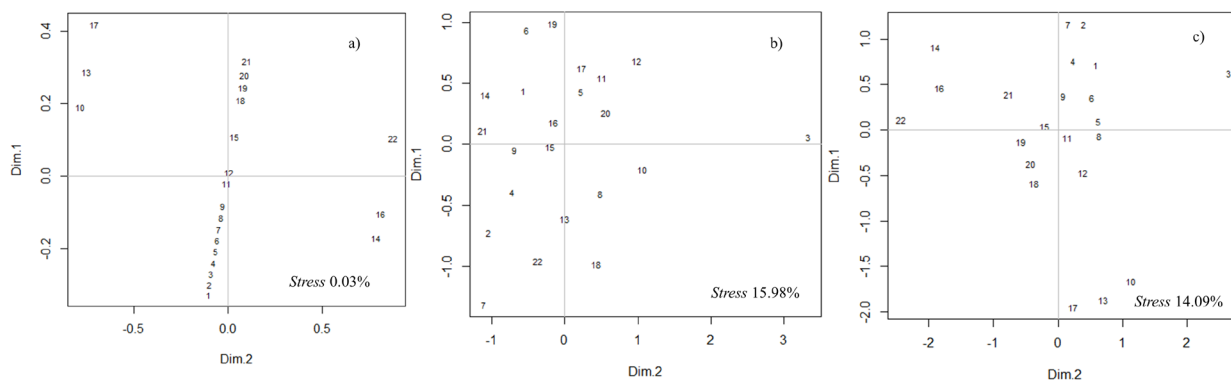


Figure 1. Graphic dispersion of 22 African mahogany (*Khaya senegalensis*) progenies by non-metric multidimensional scaling based on a) qualitative, b) quantitative and c) mixed data. Dim: dimension.

Table I. Total height (H), chlorophyll-a (Cla), chlorophyll-b (Clb), and diameter at soil level (D) of 22 *Khaya senegalensis* progenies.

Progenies	H	Cla	Clb	D
1	106.29	30.17	8.91	31.79
2	81.71	29.23	8.3	30.97
3	59.25	35.15	9.95	19.34
4	83.61	29.42	8.29	29.46
5	106.57	33.33	10.06	32.65
6	110	31.81	8.73	34.69
7	72.27	28.26	8.41	28.06
8	98.33	33.63	10.67	32.79
9	93.69	29.72	8.15	31.22
10	91.83	34.4	11	29.16
11	90.8	32.48	8.91	27.02
12	102.8	33.99	10.42	28.55
13	98.2	33.03	9.74	35.06
14	86.71	28.19	7.2	26.02
15	90.31	30.79	8.69	28.8
16	83.22	30.72	8.22	25.96
17	112.71	33.5	10.33	34.95
18	85.75	31.88	10.58	28.65
19	92.2	30.96	7.96	26.77
20	94.2	32.22	9.5	26.75
21	87	27.93	7.35	27.95
22	94.57	29.71	9.71	30.23

observing the formation of 4 distinct groups. Group 1 was formed by progenies 10, 13, and 17; group 2 was formed by progenies 14, 16, and 22; group 3 by progeny 3; and group 4 was formed by the remaining progenies, which could be clustered for being closer in the bidimensional plane, sharing similarities for quantitative and qualitative traits. The progenies of groups 1, 2, and 3 were isolated from the remainder and can be considered the most divergent. Although the progenies of groups 1 and 2 presented intermediate or high means for the variables of diameter at soil level and height, evidencing a good development in the field, compared to the remainder, these progenies also presented undesirable qualitative characters for the

production system of the species, such as the presence of lateral branches and bifurcation. Progeny 3 was isolated from the remainder for presenting a low mean for the variables of diameter at soil level and height (Table I), which is a similar result to that described in the quantitative scaling. The progenies of commercial interest were clustered in group 4 (Figure 1c), not presenting bifurcation and/or lateral branching.

DISCUSSION

The nMDS method allows the graphical visualization of individuals based on the similarity or dissimilarity of traits, so the closer the points, the more similar they are. This technique is conditioned to the good ordering of points in the bidimensional plane, and the stress value is commonly used to evaluate the percentage of unmapped information (Silva 2016). The lower the stress value, the lower are the chances of data misinterpretation (Haouari & Ferchichi 2008) since the closer to zero, the more real are the position of the points in the scaling, and the smaller is data distortion with the reduction of dimensions (Clarke & Warwick 2001). This implies a good ordering of data and, therefore, an efficient method (Haouari & Ferchichi 2008, Sturrock & Rocha 2000).

In this work, the stress values presented by the scatter plot (Figure 1) were considered excellent for qualitative data, whereas the stress for quantitative and mixed data was considered reasonable (Kruskal 1964, Sturrock & Rocha 2000). Thus, the scaling based on quantitative, qualitative, and mixed data was efficient in representing the progeny/provenance distances in the plot, indicating a good ordering.

Based on this analysis, it was possible to identify more divergent progenies, which contained phenotypes of interest for the genetic

improvement of the species. For timbers, trees with bifurcations are not suitable for commercial cultivation since the stem height is reduced, thus depreciating the wood value. Based on the mixed scaling (Figure 1c), the individuals of group 4 are considered ideal progenies for use in commercial plantations for wood production. A straight stem without lateral branches is typically commercial and is a positive indicator for selecting progenies of *Khaya senegalensis* (Carmo et al. 2018).

Lateral branches cause damage to plant tissue. The removal of lateral branches demands costs and causes the devaluation of the main commercial product, the wood. Therefore, it is interesting that the progenies of groups 1 and 3 do not have their use focused on wood production, considering that bifurcation and/or lateral branches significantly reduce the commercial value (Souza et al. 2010).

In the evaluation of juvenile plants, the variable that best predicts post-planting performance is the diameter at soil level (Ritchie et al. 2010). Thus, lower values for this character are usually related to a reduced root system, which is unfavorable for the establishment and development of plants under conditions of weed competition (Filho et al. 2012). Given the tested conditions, progeny 3 is not initially interesting for improvement.

Through forestry genetic improvement, it is possible to select superior individuals, seeking characteristics of interest (Grattapaglia & Kirst 2008) that are also important in the production sector (Maciel 2014). Thus, the selection of trees for breeding purposes must be based on quantitative and qualitative traits. Forestry productivity is influenced by the quality of the genetic material used, which is maximized when using a genetic material adapted to the local edaphoclimatic conditions.

The graphical analysis of the dispersion for mixed data showed to be the most adequate method to select progenies in the conditions of this study, evidencing a better distinction between individuals as a consequence of the higher number of groups and evidencing the importance of the fusion of qualitative and quantitative variables in this technique. J.C. Lima (unpublished data) reported that clustering analyses using matrices constructed based on Gower's distance for mixed data evidenced better dissimilarity between genotypes of *Senegalia bahiensis* than when clustered only by matrices generated with quantitative data. Gower's algorithm also expressed genetic diversity among accessions of giant coconut (Loiola et al. 2013), pepper germplasm (Moura et al. 2010), and recombinant inbred lines of common bean (Barbé et al. 2008). It is worth highlighting that the previously mentioned authors used Gower's algorithm but did not use the non-metric multidimensional scaling technique.

Based on mixed nMDS, progenies 1, 2, and 3 are not suitable for commercial plantations, for which purpose wood is the main product. However, they could be used for reforestation and/or afforestation of public squares due to the presence of bifurcation and lateral branches that give greater aesthetic value to the plant. Besides, if other studies are incorporated, these progenies may present a potential for the extraction of biochemical compounds since the species has medicinal uses, given its anthelmintic, anti-inflammatory, and anesthetic properties, among others (Pinheiro et al. 2012).

Individuals that present desirable traits should be selected for the continuity of genetic improvement programs. Therefore, with the multidimensional scaling for mixed data, it is possible to better discriminate the desirable progenies for selection within an acceptable

stress level compared to the individual analyses of qualitative and quantitative data. The formation of a higher number of groups for this graphic representation and the dispersion within clusters suggests the existence of variability for the analyzed characters, being an indicator of the possibility of gains in breeding programs (A.A. Botin, unpublished data).

CONCLUSIONS

There is phenotypic variation among half-sib progenies of African mahogany (*K. senegalensis*) grown in southern Piauí. Gower's algorithm applied to qualitative and quantitative data, and the use of non-metric multidimensional scaling are efficient to describe morphological dissimilarity between progenies, allowing to select individuals with traits of commercial interest.

Acknowledgments

To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) for the concession of the master's degree scholarship. To the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) for the financial support to the project nº 432752/2016-1. To companies: Futuro Florestal and Eco Environment Agency LTDA.

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How to cite

CRUZ AC, BARROSO PA, DIAS BAS, SILVA SC & LIMA JS. 2022. Phenotypic divergence among half-sib progenies of African mahogany. *An Acad Bras Cienc* 94: e20190990. DOI 10.1590/0001-376520220190990.

*Manuscript received on August 23, 2019;
accepted for publication on February 16, 2021*

AGNES C. CRUZ

<https://orcid.org/0000-0001-6597-7750>

PRISCILA A. BARROSO

<https://orcid.org/0000-0003-1749-1961>

BRUNA A.S. DIAS

<https://orcid.org/0000-0002-6918-6195>

SILVOKLEIO C. SILVA

<https://orcid.org/0000-0002-9975-1212>

JOAQUIM S. LIMA

<https://orcid.org/0000-0001-7775-9363>

Universidade Federal do Piauí, Campus Professora
Cinobelina Elvas, Planalto Horizonte, BR-135,
Km 3, 64900-000 Bom Jesus, PI, Brazil

Correspondence to: **Agnes Cardoso da Cruz**

E-mail: agnes1994cardoso@hotmail.com

Author contributions

Agnes C. Cruz prepared the first drafts of the manuscript, Bruna A.S. Dias; Silvokleio C. Silva; Joaquim S. Lima read and corrected the first version. Priscila A. Barroso together with Agnes C. Cruz produced several revisions of the document and worked to finalize the definitive version. All authors contributed to the article and approved the submitted version.

