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Morphological descriptors for the characterization of teak clones (*Tectona grandis* L.f.) in plantations

Jorge L. Reategui-Betancourt (Reategui-Betancourt, J.L.)[1], Daniele Aparecida A. Arriel (Arriel, D.A.A.)[1,2], Sidney Fernando Caldeira (Caldeira, S.F.)[1], Antonio Rioyei Higa (Higa, A.R.)[3], Paulo C. Flôres Junior (Flôres Junior, P.C.)[3], Stefania Palma Araujo (Araujo, S.P.)[4], Rudmilla Menezes Marques (Marques, R.M.) [5], Bruna Maria B. Corrêa (Corrêa, B.M.B.)[1] and Diego Tyszka Martinez (Martinez, D.T.)[3]

[1]Programa de Pós-Graduação em Ciências Florestais e Ambientais, Universidade Federal de Mato Grosso, Campus Cuiabá. 78.060-900 Mato Grosso, Brazil. [2]Universidade Federal de Uberlândia, Campus Monte Carmelo. 38.500-000 Monte Carmelo, Minas Gerais, Brazil.[3]Universidade Federal do Paraná. 80.060-000 Curitiba, Paraná, Brazil. [4]Auditora Fiscal Federal Agropecuário, Serviço Nacional de Proteção de Cultivares, Ministério da Agricultura, Pecuária e Abastecimento. 70.043-900 Brasilia, Brazil. [5]Graduação, Engenharia Florestal, Universidade Federal de Mato Grosso, Campus Cuiabá. 78.060-900. Mato Grosso. Brazil.

Abstract

Aim of study: The objective of this work was to identify possible morphological descriptors for teak clones, in order to support the cultivars protection process of this species.

Area of study: This experiment was carried out in 'São José dos Quatro Marcos', Mato Grosso, midwest Brazil.

Material and methods: A teak clonal test, assessing 18 clones, was evaluated at the ages of 29 and 41 months by means of 41 morphological characteristics, related mainly to the branches, leaves and trunk. The clonal test was established in a randomized block design composed by three blocks, each block containing 18 plots, one for each clone. Each plot had 36 plants, but only the innermost five individuals were selected and evaluated. The information was organized in a presence and absence matrix. Subsequently, genetic similarity measures were estimated, by means of the Jaccard index, and a clustering was performed by the Unweighted Pair Group Method using the Arithmetic averages (UPGMA) method.

Main results: A total of 26 and 28 morphological characteristic that exhibited DHS (distinction, homogeneity and stability) were identified at the ages of 29 and 41 months, respectively. Of these, 17 characteristics showed the same behavior at 29 and 41 months of age. However, it is important to emphasize that the evaluation must be performed under the same planting conditions in which these descriptors were developed.

Research highlights: These 17 morphological characteristics can compose the list of potential morphological descriptors to be used in the process of teak clones/cultivars protection.

Keywords: cultivars protection; morphological characteristics; distinction, homogeneity; stability.

Authors' contributions: Data collection, processing and analysis of the information; writing and final elaboration of the manuscript, J.L.R.B., D.T.M., and D.A.A.A. Methodological contributions and complementary review for technical support, D.T.M., D.A.A.A., S.F.C., S.P.A., A.R.H. and P.C.F.J. Textual edition of the manuscript, D.T.M and D.A.A.A. Data collection and experimental set-up, J.L.R., R.M.M., B.M.B.C., D.T.M., D.A.A.A and S.F.C. Project management, D.T.M., D.A.A.A and S.F.C.

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Supplementary material: Tables S1 to S8 accompany the paper on FS's website.

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Correspondence should be addressed to Jorge Luis Reategui Betancourt: jorgereategui91@gmail.com

Introduction

Teak (*Tectona grandis* L.f.) is a woody species belonging to the family Lamiaceae, native to Southeast Asia, mainly from Myanmar, Thailand, India, Malaysia and the Lao People's Democratic Republic (Veit, 1996; Figueiredo *et al.,* 2005). It is estimated that the natural forests of the species occupy approximately 29 million hectares and the planted forest area is between 4.35 and 6.89 million hectares (Kollert & Kleine, 2017). The beauty, workability and durability of its multiple-purpose wood, have made teak the most planted tropical wood species in the world (Kollert & Cherubini, 2012).

In Brazil, the teak was introduced in the decade of 1930 and the first commercial plantations started at the end of the decade of 1960, in the city of Cáceres, state of Mato Grosso (Cáceres Florestal, 1997; Schulli & Paludzyszyn Filho, 2010). It is estimated that in 2018, Brazil had a planted area of 93.957 ha, concentrated in the state of Mato Grosso, followed by the state of Pará (IBA, 2019). Factors such as adaptation to the country climatic conditions, availability of suitable land, high productivity and rapid growth, enabled rotation ages from 20 to 25 years (Costa, 2011; Camino & Morales, 2013). The management by high forest systems is performed with 2 to 4 intermediate thinning, with final cut at 20 to 25 years of age (Foelkel, 2013). In Central and South America, a rotation of 20 to 25 years presents a production that varies between 10 and 20 $m^3ha^{-1}year^{-1}$ (Pelissari *et al.,* 2014).

Currently in Brazil plantations with selected clones of teak have superior performance in growth in DBH (diameter at breast height), average cross-sectional area and basal area when compared with the seminal plantations (Miranda, 2013). Thence, the establishment of clonal plantations became the most adopted practice for the species in the country (Costa *et al.,* 2015).

The characterization of cultivated plant genotypes is an important step in breeding programs, germplasm conservation and is essential in the process of protecting cultivars. The increase in planted area and the high value that teak wood has on the international market aroused in many Brazilian foresters and breeders the interest in seeking mechanisms that allow the intellectual protection of the teak clones developed by them.

Brazil is a member country of the UPOV (Union for the Protection of New Varieties of Plants) and the guidelines for the process of crop protection in the country were established by the Ministry of Agriculture, Livestock and Supply by the Cultivar Protection Law, No. 9,456, of April 25, 1997, regulated by the Decree No. 2,366, of November 5, 1997. This law guarantees intellectual property rights to the creators of a new cultivar. According to this law, the protection of a new cultivar must be result of genetic improvement and must be clearly differentiable from other cultivars through characteristics called morphological descriptors that guarantee its distinction, homogeneity and stability (DHS) through successive generations, creating an identity for each genotype. Thus, for each species, morphological descriptors must be selected to guarantee their distinction of different cultivars to be protected (Aviani, 2011; Santos & Pacheco, 2011).

Although it is an important species in the Brazilian economy, teak still does not have minimum descriptors established by the Ministry of Agriculture, Livestock and Food Supply for cultivars protection in the country, as well as in other countries around the world.

Studies related to morphological characterization have already been developed in teak clones at different ages, allowing differentiation among groups of genotypes and origins, besides identifying the genetic variation (Gunaga *et al.,* 2013; Miranda, 2013; Alcântara *et al.,* 2016; Baretta, 2016; Chimello *et al.,* 2017). However, it has not yet been possible to establish a table of morphological descriptors for the differentiation of genotypic based on the morphological characteristics used, as they did not meet the DHS test. Thus, the objective of this study was to evaluate morphological descriptors to differentiate teak clones in plantations, in the southwest region of the state of Mato Grosso, in order to propose morphological descriptors to support the cultivars protection process in Brazil and in the world.

Materials and methods

Morphological descriptors

The trial is located in Rancho Alegre Farm, located in the municipality of São José dos Quatro Marcos, southwestern region of the state of Mato Grosso. The clonal test was established in January 2015 and is installed in a completely randomized block design (DBC) with three blocks, 18 clones (treatments) (Table 1), and 36 trees per plot (square plots of 6 rows x 6 columns), with spacing of 4 m x 4 m. A pruning was carried out in the first year after planting. All the genotypes are imported from other countries and were selected by teak-producing companies in Mato Grosso. Some of them are commercial and others are in the testing phase. For the survey of morphological descriptors, in each plot, five internal trees from the central position were selected and evaluated, totaling 15 trees per clone. Whenever a tree died or had a severe physical damage,

Table 1. Evaluated genotypes and their respective origins

Origins	Clones
Solomon Islands	1,2,4,7,8,10,15
Malaysia	9,14
India	3
Indonesia	5
Laos	6,11,12,13,16,17,18

the next tree in the plot was evaluated, avoiding the borders. The evaluations were carried out in June 2017, at 29 months of age, and in June 2018 at 41 months of age, before the fall of the leaves.

Data Collection

The data collection was performed by trained staff to analyze and evaluate the morphological characteristics previously established in former visits to the experiment. During the data collection, new characteristics that could potentially be used as morphological descriptors were identified. Thereby, 41 qualitative and quantitative characteristics related mainly to the branches, leaves and trunk were evaluated (Table 2).

To evaluate the characteristics such as petiole, pubescence in the lower face, intensity of the color green on top and bottom, size, margin, undulation of the margin, venation and brightness, branches were collected between 40% and 60% of the total height of the tree. The collected branch was located in the intermediary part of the crown, i.e. below the terminal position of the main branch and above the first branches at the base of the

Table 2. Morphological characteristics evaluated in teak clonal test at 29 months and 41 months of age

Number	Characteristic	Levels of expression	Analysis Methods
1	Trunk: crown habit	Fluted, cylindrical, oval and tabulate	VG
2	Trunk: number of internodes per linear meter (measure from 1m above the ground)	Number of internodes	VG/MI
3	Trunk: distance between nodes in 1 m (Measure from 1m above the ground)	Equal to 12 cm, higher than 12 cm, lower than 12 cm	VG/MI
4	Trunk: insertion angle of the branches	Acute, Right, obtuse	VG
5	Trunk: intensity of gray color	Light, medium, dark	VG
6	Trunk: color of inner bark	Whitish, yellowish, light green, dark green	VG
7	Trunk: suckers	Absent, present	VG
8	Trunk: spots	Absent, present	VG
9	Trunk: intensity of the brownish color of bark	Light, medium, dark	VG
10	Trunk: persistence of bark	Low, medium, high	VG
11	Trunk: bark drop	No drop, cracks, boards, scales	VG
12	Crown: density	Low, medium, high	VG
13	Branch: tropism	erect, curved, pending	VG
14	Branch: shape in cross section	Cylindrical, oval	VI
15	Branch: pubescence	Absent, present	VG
16	Branch: leaf position	In every branch, only at the apex	VG
17	Apical branch: leaf position	Throughout the apical branch, only at the apex of the apical branch	VG
18	Apical branch: insertion of branches	Absent, present	VG
19	Branch: sprouting	Absent, present	VG
20	Leaf: phyllotaxy	Opposite and decussate	VG
21	Leaf: attitude	Erect, horizontal, pending	VG
22	Leaf: petiole	Absent, present	VG
23	Leaf: petiole length (cm)	Small, medium and large	VG
24	Leaf blade: length (cm)	Short and long	VG/MI
25	Leaf blade: width (cm)	Narrow and wide	VG/MI
26	Leaf blade: ratio length/width (cm)	Small and large	VG/MI
27	Leaf blade: pubescence on the upper face	Absent, present	VG
28	Leaf blade: pubescence on the lower face	Present and Absent	VG
29	Leaf blade: consistency	Membranaceous, coriaceous, cartaceous	VG
30	Leaf blade: the intensity of the color green on the upper face	Light and dark	VG
31	Leaf blade: the intensity of the color green on the lower face	Light and dark	VG
32	Leaf blade: shape	Elliptical, oval, triangular, obovate	VG
33	Leaf blade: shape of the tip of the apex	Acuminate, acute, cuspidate, Mucronate	VG
34	Leaf blade: shape of the basis	Oblique, cuneate, obtuse, attenuated, truncated	VG
35	Leaf blade: margin	Dentate, whole	VG
36	Leaf blade: margin undulation	Low, medium, high	VG
37	Leaf blade: main vein	Does not touch the margin, touches the margin,	VG
38	Leaf blade: veins	Secondary, tertiary, quaternary,	VG
39	Leaf blade: venations	Does not touch the margin, touches the margin,	VG
40	Leaf blade: brightness	Absent, present	VG
41	Inflorescence	Absent, present	VG

Where: (MI) individual measurements of a certain number of plants or their parts; (VG) visual assessment from a simple observation of a group of plants or parts of plants; (VI) the visual evaluation from the observation of an individual plant or parts of plants.

crown. A leaf sheet located in the middle portion of the branch was evaluated (Fig. 1). The heights of the trees ranging from 5.50 m to 6.50 m for 21 months of age and from 9.5 m to 10.5 m for 41 months of age. These heights are compatible with plantations of the species in Brazil (Miranda, 2013; Baretta, 2016). For the evaluation, the leaf was completely expanded and had no physical damage.

The characteristics, such as leaf attitude, shape of the apex, insertion angle of the branches, leaf position on the branch and apical branch, tropism, density, and sprouting were evaluated with the general observation of the crown of the tree, with a record of the prevailing level of expression. The trunk characteristics, such as number of internodes per linear meter and distance between the nodes, were measured from 1 m from the soil surface up to 2 m. The other trunk characteristics as the color of the inner bark, stains, intensity of the brownish color of the bark, persistence of the bark and bark drop, were valued at 1.30 m height in relation to the soil surface. The leaf morphological characterization, such as disposition, consistency, shape of the leaf blade, and margin and shape of the apex was performed according to Vidal & Vidal (2003).

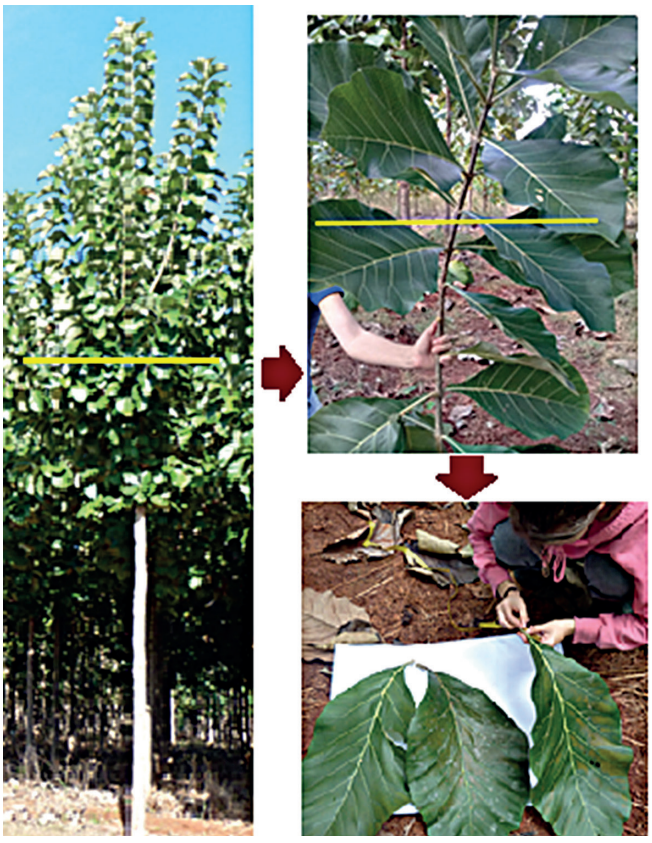

Figure 1. Branches collection process and morphological characterization of leaves from the middle part of the branch. The yellow line indicates the location where the samples were collected (leaves and branches).

Table 3. Example of a table for completion qualitative morphological descriptors and quantitative characteristics, mainly of the leaf blade

Characteristic	Identification of characteristic	Description code	Cultivar code
Leaf blade: shape	Elliptical Oval Triangular Obovate	1 2 3 4	| |
Trunk: bark color	Light Medium Dark	1 2 3	| |
Length (cm)	Short Long	1 2	| |
Width (cm)	Narrow Large	1 2	| |
Length/width ratio (cm)	Small Large	1 2	| |

Evaluation of the descriptors

To facilitate the evaluation of various characteristics in the field, a scale of sequential codes with values ranging from 1 to 9 was developed. For example, for the characteristic "Leaf blade: Shape"; value 1 for "elliptical"; value 2 for "oval"; value 3 for "triangular"; and value 4 for “obovate" (Table 3). Thus, for each clone, the code that corresponded to each of the evaluated characteristics was noted.

The characteristics whose level of expression was not homogeneous within the same clone were marked with the letter (X).

To evaluate the quantitative morphological characteristics, such as length, width and length/width ratio of leaf blade, the average $(\bar{x})$ for each characteristic was calculated by clone. Subsequently, on the basis of the overall average (average of all clones), for each characteristic two classes were established: (1) when the value observed was lower than the overall average and (2) when the value observed was higher than overall average (Table 3). As well as for qualitative characteristics, the clones that showed no homogeneity for the quantitative characteristics were marked with the letter "X".

Based on the selected morphological characteristics (with the same pattern within the same clone), each genetic material received a code corresponding to its phenotype for that characteristic. Subsequently, these codes were grouped by clone, creating identification (numeric code) for each clone.

Genetic similarity and cluster analysis

In this analysis, for each age (29 and 41 months), only the morphological characteristics that showed distinction among the clones and homogeneity within the individuals (that have not received the letter "X", as explained in the

last section) were used. The binary matrices of presence (1) and absence (0) were arranged according to the level of expression of the characteristics presented by each clone (Table S1 and S2 [suppl.]).

After obtaining the binary matrix, the genetic similarity among the evaluated clones was calculated by means of the Jaccard similarity index (SJ).

$$SJ\,\frac{c}{a+b-c}$$

Where:
a = number of morphological characteristics occurring in clone 1;
b = number of morphological characteristics occurring in clone 2;
c = number of common morphological characteristics in the two clones.

Subsequently, the clustering analysis by the UPGMA method (unweighted pair group method with arithmetic mean) was performed by means of the program Past 3.x (Hammer *et al.,* 2001). The cut-off point in the dendrogram was made at a distance of 0.5 to obtain a better determination of the number of conformed groups.

Results

Selected descriptors for each age

From 41 evaluated characteristics, 26 and 28 of them allowed the distinction among clones, at 29 and 41 months of age respectively (Table S3 and S4 [suppl.]). These characteristics presented distinction among the clones and homogeneity within individuals of the same clone (got they have the same code). For the two ages, these are the characteristics for the trunk: insertion angle of the branches, intensity of the color gray, brown color intensity of the bark, inner color of bark, suckers, persistence of bark, and bark drop; for the branch: leaf position, tropism, and sprouting; for the apical branch: leaf position; for the crown: density; for the leaf: attitude, and petiole; for the leaf blade: length, width, length/width ratio, pubescence on the lower face, intensity of the color green on the upper face, intensity of green color on the lower face, shape, shape of the tip of the apex, margin, undulation of the margin, venation, and brightness. The characteristics "presence of inflorescence" and "spots in the trunk" appeared only at the older age.

There were characteristics that were not homogeneous and others that did not allow distinction among the genetic materials. These were the characteristics for the trunk: crown habit, number of internodes per linear meter, and distance between nodes in 1 m; for the branch: shape in cross section, and pubescence; for the apical branch: insertion of branches; for the leaf: phyllotaxy, and petiole length; and for the leaf blade: pubescence on the upper face, consistency, shape of basis, main vein, and veins. Therefore, they were discarded as morphological descriptors because they did not meet the DHS test. In table S5 [suppl.], the authors show how these characteristics were estimated and evaluated.

Through the "numeric code" created for each clone, it was possible to observe that some characteristics have the same pattern for some clones. However, all clones could be differentiated by at least one characteristic (Table 4).

Table 4. Morphological descriptors code that allows the distinction among the clones of *Tectona grandis* L.f. at 2 evaluation times and of the 17 morphological characteristics that met DHS at 29 and 41 months of age

	Age and clone code		
Clone	**29 months**	**41 months**	**29 and 41 months of age**
1	123211211113111111111232112	133212121111321111112321122	12112113111123212
2	133213211113211111111112222	133211321111311111111122222	12132113111111222
3	133212222113322222211111121	133212221111332222222111121	12122113222211121
4	232211211213211111121232112	232212121121321111121232112	22112213111123212
5	223112212113221111111122112	233112221111322112111221121	21122113211112212
6	322213122323322222111111121	322212311132332212111111212	32131323222211121
7	221323122122222222211122111	221321311212222222222221222111	23231122222212211
8	221213112113221221111122111	223211311111322121121221122	22131113212112212
9	233212211113121111111112311	233211221111322112111122121	22122113211111212
10	133313111113211111111122212	133311311111321112111222122	13131113111112212
11	113423122111322222212112111	111421312211122221222122112	14231111222211211
12	222423122221322222222112111	211421312222132222222121121111	24231221222211211
13	122423122121322222211112111	123421312212132222211121111	14231121222211211
14	233313122113211111211122112	233312311111321111222122112	23131113111212212
15	133212212113221111111112122	133211221111322111121122221	12122113211111222
16	211413122111222222211112111	211411312211122222211121112	24131111222211211
17	211423122121322222222122211	211421311212132221222122211	24231121222212211
18	222413122122222222222122111	222411311212222221222122111	24131122222212211

Genetic similarity and cluster analysis for clones at age 29 months

For the 26 morphological characteristics at the age 29 months, at a similarity distance of 0.5 the formation of five groups was observed (Fig. 2a): group one (clones 7, 11, 12, 13, 16, 17 and 18), group two (clone 8), group 3 (clones 3 and 6), group four (clones 1 and 4), and group 5 (clones 2, 5, 9, 10, 14 and 15). Clone 8 presented lower similarity in relation to the other genotypes, with a distance of 0.45. The most similar clones in their morphological characteristics are clones 11 and 13 and clones 2 and 10, presenting similarity of 0.73 (Table S6 [suppl.]).

The clones 11 and 13 differ by the trunk characteristics, intensity of gray color and intensity of the brown color of the bark, the bud, sprouting, leaf blade, and intensity of thee green color on the lower face. Clones 2 and 10 differ by the characteristics of the trunk, inner color of bark and

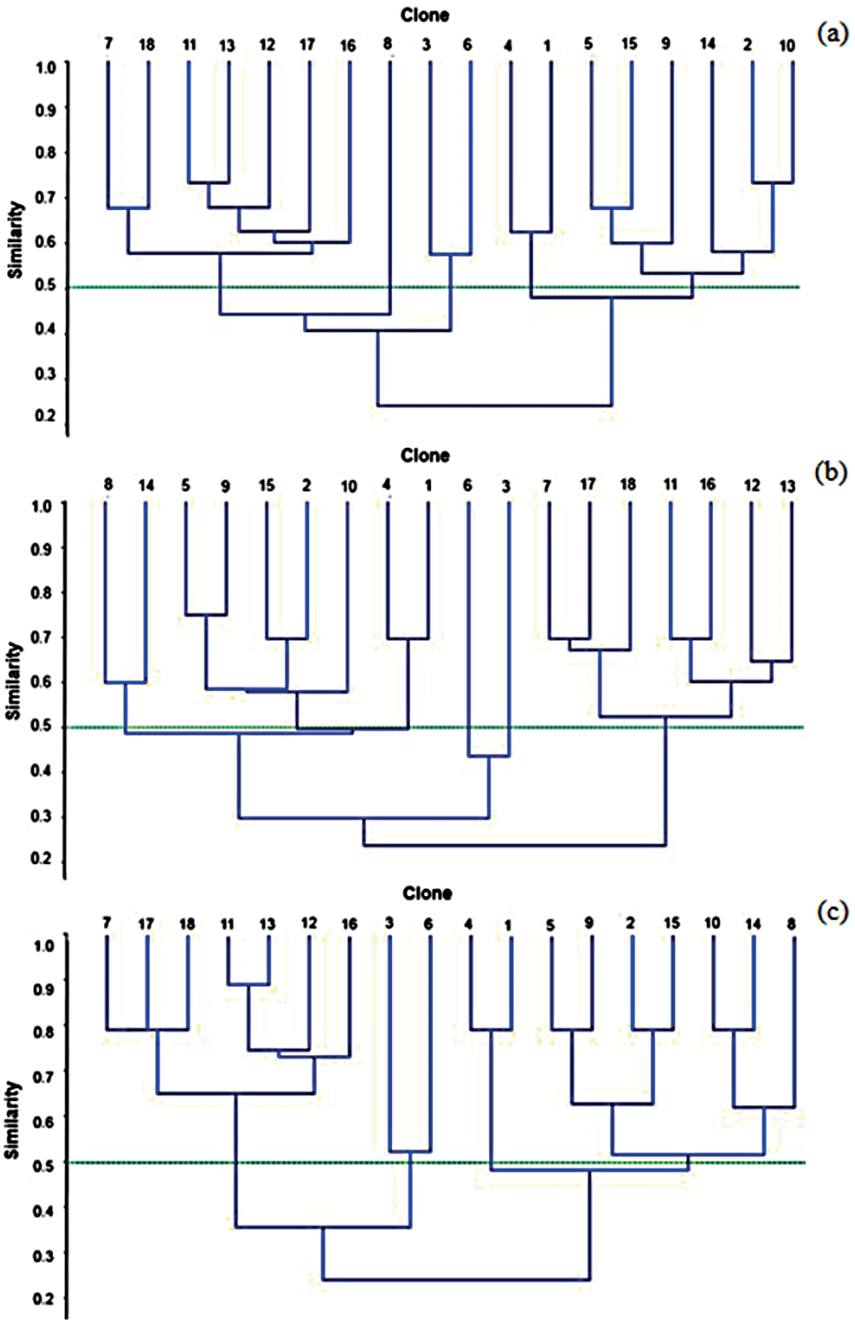

Figure 2. Dendrogram of similarity of *Tectona grandis* L.f. clones, upon the UPGMA clustering method, based on the Jaccard similarity index. The similarity was obtained by evaluating 26 and 28 morphological characteristics for the ages of 29 (a) and 41 (b) months respectively and for the 17 characteristics that exhibited DHS and remained at the same ages of evaluation (c).

bark drop, and leaf blade shape from of the tip to the apex and venation.

Genetic similarity and cluster analysis for clones at age 41 months

For the 28 morphological characteristics at age 41 months, it is also observed that all the clones have distinction among them (Fig. 2b). At a similarity of 0.5, it is possible to identify six groups: group one (clones 8 and 14), group two (clones 2, 5, 9, 10 and 15), group 3 (clones 4 and 1) group four (clone 6), group five (clone 3) and group 6 (clones 7, 11, 12, 13, 16, 17 and 18). The genotypes that showed fewer similarities at this age are clones 3 and 6 with a distance of 0.43. Greater similarity occurred between clones 5 and 9, with a distance of 0.75 (Table S6 [suppl.]), which are differentiated by the following trunk characteristics: insertion angle of the branches, color of inner bark, and spots on the trunk; leaf blade: shape from the tip to the apex, and undulation of the margin.

Selected descriptors for both ages

From 41 characteristics evaluated, only 17 showed the same behavior for both ages and met the requirements of the DHS test and, thus, can be considered morphological descriptors (Table S7 [suppl.]). These characteristics are related to the trunk: the insertion angle of the branches, color of inner bark and presence of suckers, persistence of bark, and bark drop; the branches: tropism and sprouting in branches; the canopy: density; the leaf: petiole; and the leaf blade: length, width, pubescence on the lower face, shape, shape from the tip to the apex, margin, venation, and brightness.

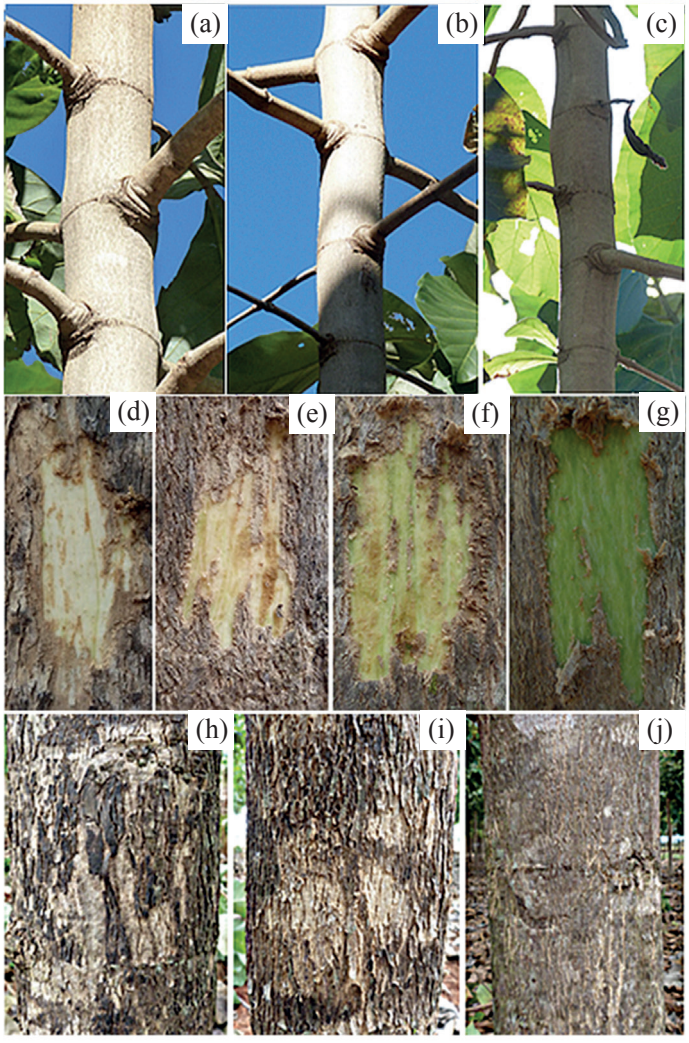

Figure 3. Insertion angle: acute (a), right (b) and obtuse (c) branches; color of the inner bark of the trunk: whitish (d), yellowish (e), light greenish (f) and dark greenish (g). Persistence and bark drop of the trunk: low with drop (h), medium with drop (i) and high without drop (j).

Just as it happened for each age separately through the "numeric code" created for each clone, it was possible to observe that some characteristics have the same pattern for some clones. However, all clones could be differentiated by, at least, one characteristic (Table 4).

For the 17 characteristics that enabled distinction at both ages, each of them was described below: the insertion angle of the branches was obtuse only for the clone 6, while clones 1, 2, 3, 10, 11, 13 and 15 showed acute angle and the other clones showed the right angle (Fig. 3a-c).

The color of the inner bark of the trunk for clone 5 was whitish; for clones 7, 10 and 14, it was light greenish; clones 11, 12, 13, 16, 17 and 18 had dark greenish coloration; and the other clones were distinguished by the yellowish color (Fig. 3d-g).

The persistence of the bark was low for clones 1 and 4, medium for clones 3, 5, 9 and 15, and the other showed high persistence of the bark. For the bark drop, the clones 1, 2, 3, 4, 5, 9 and 15 were in the form of cracks, and the others had no drop (Fig. 3h-j).

In terms of tropism for the branch, clone 6 showed pendant tropism, clones 4 and 12 were bent, and the other clones showed erect tropism (Fig. 4a-c). For the density of the crown, clones 11, 12, 13, 16 and 17 showed low density; clones 7 and 18 had a medium density; and the other clones showed high density (Fig. 4d-f).The presence of sprouting in the branches was observed for clones 6, 7, 12, 13, 17 and 18 (Fig. 4g). Presence of suckers was observed in clones 7, 11, 12, 13 and 17 (Fig. 4h).

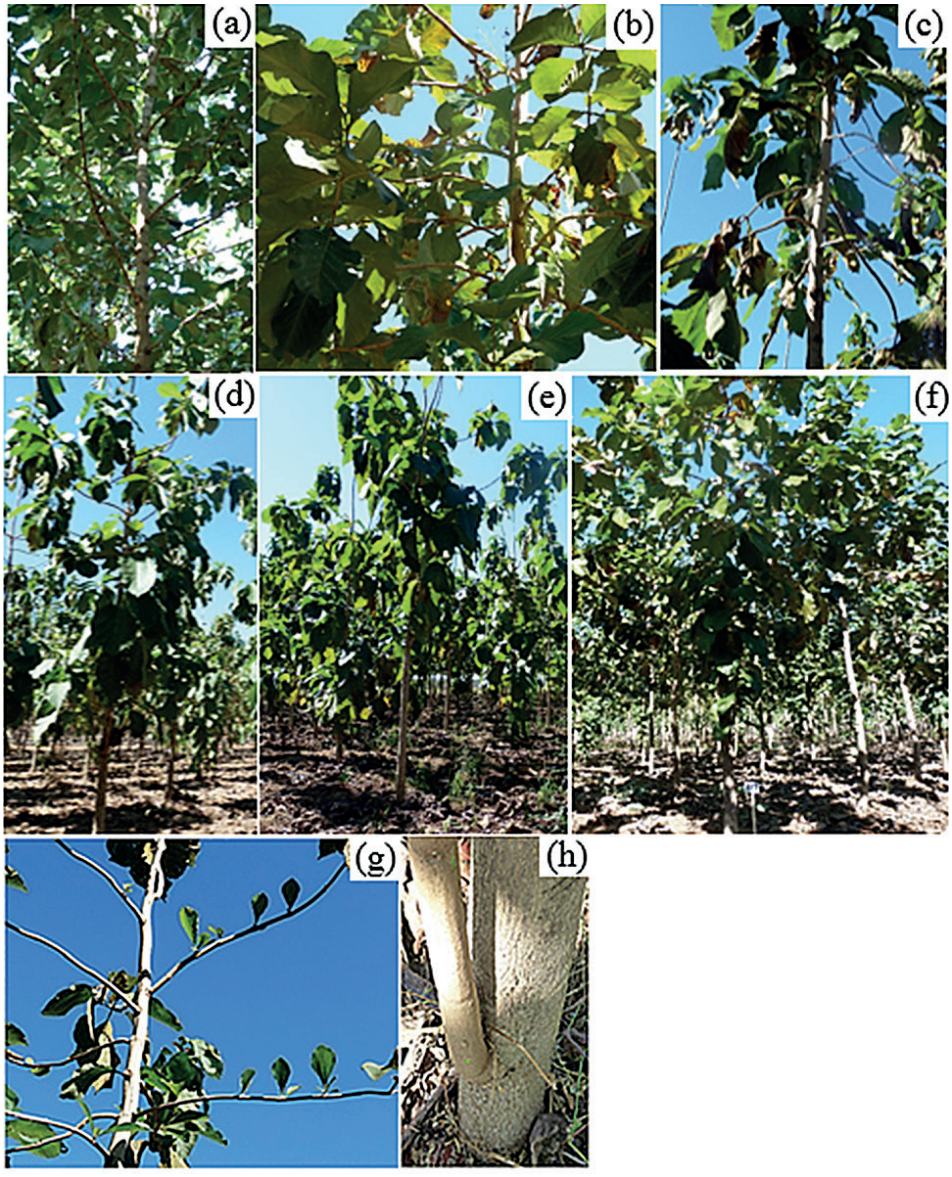

Figure 4. Tropism: erect branch (a), curved (b) and (c) pending; crown density: low (d), medium (e) and high (f); presence of sprouts in the branch (g); presence of suckers in the trunk (h).

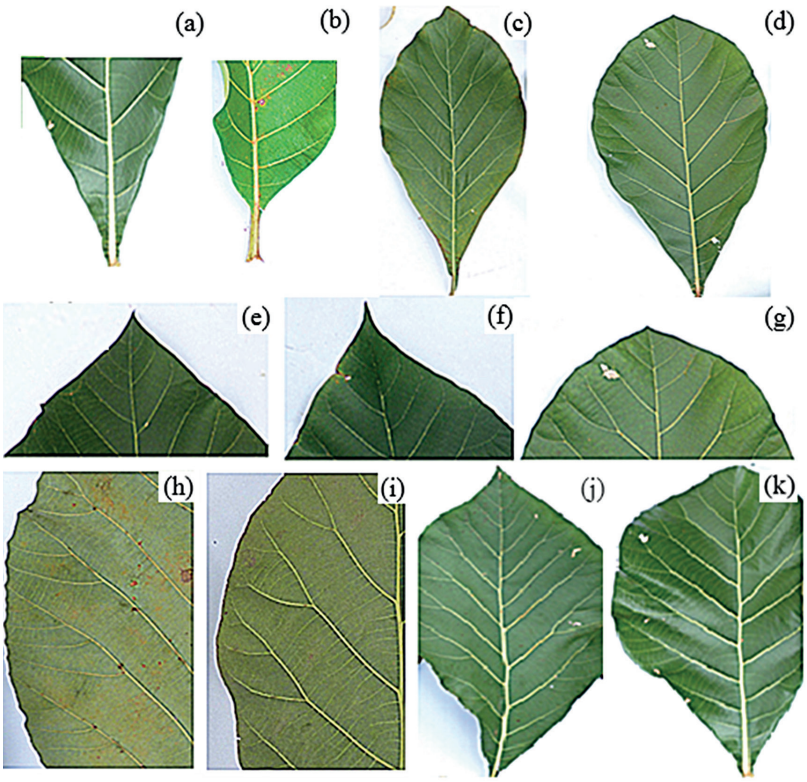

Figure 5. Petiole missing (a) and present (b), leaf in elliptical form (c) and obovate (d), acute apex (e) cuspidate (f) and mucronate (g); margin of the leaf blade: dentate and venation touch the margin (h); the margin of the whole leaf blade and venation does not touch the margin (i); brightness absent (j); and present (k).

The petiole was present for most of the clones, and was absent for clones 1, 2, 4, 10 and 14 (Fig. 5a,b). The length of the leaf blade was short for clones 1, 2, 4, 5, 8, 9, 10, 14 and 15, and the others had long length. The length of the leaf blade ranged from narrow for clones 1, 2, 4, 5, 9, 10, 14 and 15 to large for the other genotypes. The shape of the leaf blade, for most of the clones, presented an elliptical shape and only clones 1 and 4 showed obovate shape (Fig. 5c,d). The shape of the apex of the leaf blade ranged between mucronate for the clones 1 and 4, cuspidate for clones 5, 7, 8, 10, 14 and 17, and acute for the others (Fig. 5e-g).

The margin of the leaf blade for most clones was whole, and for clones 3 and 6, it was dentate; whilst the venation of the leaf blade for clones 2, 3, 6 and 15 touches the margin of the leaf blade (Fig. 5h,i). Another characteristic that also enabled distinction among clones was leaf brightness, and it was present in nine clones (Fig. 5j,k).

Genetic similarity and cluster analysis for clones using the descriptors with the same pattern at both ages

Based on the clustering analysis for the 17 characteristics considered as morphological descriptors, it is observed the formation of four groups (Fig. 2c) at a similarity of 0.5: group one (clones 7, 11, 12, 13, 16, 17 and 18), group two (clones 3 and 6), group 3 (clones 1 and 4), and group four (clones 2, 5, 8, 9, 10, 14 and 15). The group 1 (clones 7, 11, 12, 13, 16, 17 and 18) grouped most of the clones from Laos at both ages. For the others, it seems that there is some association, with the proximity of some clones from the Solomon Islands. The grouping is also associated with the majority of the clones as to their origin, mainly those from Laos (clones 11, 12, 13, 16, 17, 18) and the Solomon Islands (clones 1, 2, 4, 8, 10, and 15), and is very different among them (Fig. 2c).

According to similarity matrices of morphological characteristics (Table S8 [suppl.]), the genotypes that showed high similarity have specific characteristics which allow the distinction. For example, clones 11 and 13 from Laos are the closest, with similarity of 0.88 and differed only in the characteristic of the branch sprouting. There are also very different genotypes such as clones 1 and 12, which are of the same origin in the Solomon Islands with a similarity of 0.06. These clones share only two characteristics: the margin and venation of the leaf blade.

Clones 3 and 6 from India and Laos, respectively, also showed low similarity, at a distance of 0.52, distinguished by the characteristics of the trunk (insertion angle of the

branches, persistence of bark and bark drop) and of the branch (tropism and sprouting).

Discussion

Cultivars or new varieties of plants are the result of intensive breeding programs of long duration and high investments. Especially in the case of timber species, such as teak, the selection of the best genetic material occurs in advanced ages, which may occur after decades. Thus, the establishment of descriptors that support the protection process and, at the same time, bringing information about the genetic variability of elite clones is a demand of foresters who work with this species. Upon the methodologies and analyzes performed in this study it was possible to identify 26 and 28 morphological characteristics that can be used as descriptors at ages 29 and 41 months of age, respectively. Of these, 17 characteristics showed the same behavior at both two ages. To the best of our knowledge, this is the first study to list morphological descriptors that enable the intellectual protection of teak clones worldwide.

Currently, the use of molecular markers has been the main approach used to study genetic diversity and characterize teak genotypes in Brazil and in the world (Fofana *et al.,* 2009; Verhaegen *et al.,* 2010; Ansari *et al.,* 2012; Alcântara & Veasey 2013; Vaishnaw *et al.,* 2014; Thwe-Thwe-Win *et al.,* 2016; Chimello *et al.,* 2017; Giustina *et al.,* 2017; Chaudhari *et al.,* 2018; Prasetyo *et al.,* 2020). Regarding to use of morphological markers, studies are less recurrent (Alcantara & Souza, 2007; Gunaga *et al.,* 2013; Alcântara et al, 2016; Chimello *et al.,* 2017) and they have no success in differentiating all the materials evaluated, which is essential for the protection process. However, this work, based on the DHS test, could distinguish all the genotypes evaluated.

In Brazil, there are morphological descriptors for other forest species. The total of 37 morphological descriptors were identified and registered for eucalyptus (*Eucalyptus* spp.), 44 descriptors for pines (*Pinus* spp.), 27 descriptors for rubber tree (*Hevea* Aubl.) and 35 descriptors for Australian red-cedar (*Toona ciliata* M. Roemer var. australis) (MAPA, 2019). For black wattle (*Acacia mearnsii* De Wild.), it was possible to identify 25 characteristics to compose a list of descriptors that are minimum and recommended for the process of plant protection (Flôres Junior, 2015; Flôres Junior *et al.,* 2018). Therefore, the number of descriptors identified in this study is lower than that observed for other forest species.

Regarding the use forest species mentioned above, the morphological descriptors involved different stages of the plant ontogeny, from seeds to mature plants (MAPA, 2019). For example, for eucalyptus seedlings, plants with 2 to 3 years of age and 5 years of age were used to elaborate the descriptors. For pines, descriptors extend from seeds, seedlings, plants aged 11 months to 7 years of age. For rubber tree, the characteristics identified were in plants with 1.6 years of age and in mature trees with a minimum of 5 years of age. The Australian red cedar was observed in seedlings of 10 to 14 months of age and trees of 2 and 4 years of age. The black wattle seed and individuals were evaluated at 15 and 24 months of age (Flôres Junior, 2015; Flôres Junior *et al.,* 2018). Therefore, it is observed that evaluating different stages of plant development is important to determine the morphological descriptors, since the number of potential characteristics to be evaluated to produce the descriptors is higher. Thus, studies with teak seedlings are being conducted in order to increase the number of descriptors for the species (Reategui-Betancourt, 2019).

The narrow genetic basis of *T. grandis* plantations in the country is a factor that may also have limited the number of descriptors observed for the species (Alcântara & Veasey 2013; Giustina *et al.,* 2017). For other species, such as *Eucalyptus* spp., the descriptors were developed involving a greater number of species *(E. grandis, E. urophylla, E. globulus, E. pellita, E. robusta, E. camaldulensis, E. saligna, E. tereticornis, E. viminalis, E. maidenii, E. deanei),* in addition to involving genotypes from hybrid combinations (MAPA, 2019).

The descriptors published for the forest species are based on the morphological characteristics of leaves, trunks, branches, flowers, fruits, seeds and plants in general. Only for Australian red cedar the leaf, the foliole, the trunk and plant traits were evaluated (MAPA, 2019). For teak, only morphological characteristics of leaves, trunk and branches were evaluated, but it was suggested the possibility of using the inflorescence morphological characteristics. However, not all the clones presented inflorescence at the evaluated ages, which hindered the analysis. Thus, we decided not to use this part of the plant for the elaboration of the descriptors, to be able to identify them at earlier ages, and to enable the protection of teak genotypes earlier.

For some clones in the field, there are some characteristics that varied from one year to the next; therefore, they did not remain stable over time. These characteristics were the intensity of the gray color of the trunk bark, leaf position in the apical and lateral branches, insertion of branches in the apical branch, the branch pubescence, leaf attitude, intensity of the color green leaf blade on the upper and lower face, and undulation of the leaf blade margin. This variation can be related to the age of the genetic materials or environmental factors and the phenotypic plasticity manifestation, i.e. response that has the same genotype in its phenotype owing to environmental changes (Pigliucci, 2001; Pigliucci & Preston, 2004).

Some characteristics seem to be related, such as the color of the inner bark of the trunk, which ranged from whitish to yellowish in clones that had higher leaf density of the crown and dark green for the clones with lower

density. The density of the canopy directly influences the external color of the bark by the luminous intensity received, with light colors being those that provide the plant with greater degree of adaptation to tropical conditions by reflecting light and avoiding overheating of the coating tissue (Appezzato-da-Glória & Carmello-Guerreiro, 2009). This process causes the epidermis to change in the maturity of the suberous cells (Silveira, 2004; Cutler *et al.,* 2011; Taiz & Zeiger, 2015; Appezzato-da-Glória, & Carmello-Guerreiro, 2009), the phelloderms being the places where the chloroplasts, responsible for the green color of the stems developing the photosynthetic capacity in the plant, contained (Appezzato-da-Glória, & Carmello-Guerreiro, 2009). It can be assumed that the trunk exposure to direct contact with sunlight influences the tone of the inner bark. In this sense, the spacing can be another decisive parameter for identification or differentiation of these or other morphological characteristics in the plant, due to the influence of the amount of light that enters the interior of the crops. The bright variations produced in plants can be observed and influenced in leaves, stem, roots and also in fruits (Pigliucci & Preston, 2004). Thus, the descriptors analysis at same age and planting conditions, mainly in relation to the quality of the site, is essential for the descriptors validation.

According to the observations made in the dendrograms for the 17 characteristics that showed DHS, clones from the same geographic origin sometimes showed a high and sometimes low similarity. For example, clone 3, which comes from India, and clone 6 from Laos, showed low similarity, whereas clones 11 and 13 from Laos showed high similarity.

The existence of clones with high similarity shows that few characteristics will be able to distinguish them, becoming keys within the process of cultivar/clone protection. In this way, the similarity analysis, in addition to providing information on the genetic variability of the genotypes, also enabled the separation of all materials, a fact that supports the protection of cultivars for teak. These differences observed and the divergences between genotypes can be explored and combined for genetic experiments (Cardoso *et al.,* 2007; Alcântara & Veasey, 2013; Chimello *et al.,* 2017; Flôres Junior *et al.,* 2018). Finally, as the present study is the first to establish a table of morphological descriptors for teak, we expect that our results can assist the species protection process in Brazil and worldwide.

Conclusion

It was possible to identify morphological descriptors to differentiate clones of *T. grandis* in plantations in Brazil. The total of 26 morphological characteristics was identified at 29 months of age and 28 morphological characteristics at 41 months of age. Of these, 17 characteristics showed the same behavior at 29 and 41 months of age. Therefore, it is suggested that these 17 characteristics that showed DHS, and remained stable at ages, can be used as descriptors to enable those interested in obtaining the intellectual property of genotypes for a greater period of time for the materials evaluation.

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