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Identification of morphological descriptors for characterization of teak (*Tectona grandis* L. f.)

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Abstract
The implantation of teak (*Tectona grandis*) in Brazil has increased in recent years; however, morphological descriptors of teak are still lacking. Thus, vegetative and reproductive materials of teak were collected from Brazil (Cáceres) and compared with different provenances in several commercial and experimental plantations: the Solomon Islands, Indonesia and Thailand. A survey of thirteen quantitative morphological traits of leaves, inflorescences and flowers and qualitative traits, such as leaf shape and type of leaf trichome, was performed at the ESA Herbarium. The quantitative traits included petiole length; leaf length; leaf width; inflorescence length and width; calyx lobe and corolla lobe length; fillet, anther and ovary length; and number of sepals, petals and anthers. Of the thirteen quantitative traits, the Indonesia provenance presented ten traits that were similar to Cáceres. The Thailand materials presented nine similar traits, whereas the Solomon Islands materials were the least similar, possessing only seven similar traits. In addition, two qualitative traits were considered: leaf shape and trichome type. Of the trichomes, the provenance that differed the most from the others was the Solomon Islands provenance, which presented arachnoid trichomes. This preliminary study describes quantitative and qualitative morphological traits of teak that can inform future studies seeking to establish potential descriptors.
Key words: Morphological traits; Trichomes; Cultigens protection.

Introduction

Teak (*Tectona grandis* L. f. - Lamiaceae according to APG 2009) is a tree species of great economic interest, representing 4% of all of the wood that is marketed worldwide and primarily known for its quality in the furniture and naval industries (Rondon Neto 1998; Krishnapillay 2000).

According to FAO (2006), in Southeast Asia, teak can reach an MAI (mean annual increment) of 4-6 m^3/ha/year during a cycle of 50-80 years in semi-natural forests. In Brazil, teak is suited to the region of Cáceres, Mato Grosso state, reaching an MAI of 10-15 m^3/ha/year during a cycle of 25 years (Chaves 2013 and references therein). The favorable climate for teak, tropical and humid with rainy summers and dry winters, contributed to the adaptation of this species to the Cáceres region (Matricardi 1989). In Brazil, the large-scale teak plantations in Mato Grosso, Pará and Roraima increased 6.5% compared to 2011, reaching 67,329 ha of total planted area in 2012 (ABRAF 2013), primarily through seeding.

Although the market for teak has increased in recent years, no teak descriptors have been established in the international databases, such as the International Plant Genetic Resources Institute (IPGRI) and the Brazil Ministry of Agriculture (MAPA).

According to IPGRI (2006), there are many definitions of descriptors in genetic resource documentation; three of these descriptors are defined as follows: passport descriptors, which provide basic information for use in the general management of the accession and describe parameters that should be observed when the accession is originally collected; characterization descriptors, which enable the easy and quick discrimination between phenotypes and are generally highly heritable, easily seen by the eye and equally expressed in several environments; and evaluation descriptors, which can be susceptible to environmental differences but are generally useful in crop improvement and may involve complex biochemical or molecular characterization. These evaluation descriptors include yield, stress susceptibilities, and biochemical and cytological traits.

A few studies of teak descriptors are available in the literature: an important study published by Keiding (1985), who described general botanical traits for teak; the work of Baillères and Durand (2000), who studied the main wood quality factors related to technological characteristics; the work of Vasudeva et al. (2004), who described the variations in the floral traits of teak; and the most recent work of Sreekanth et al. (2014), who studied genetic and morphological variation in natural teak populations.

According to Keiding (1985), teak wood is dense and gray or brown; the leaves are deciduous, opposite, and elliptical or oval, with a length of 25-50 cm and a width of 15-35 cm; the adaxial face is rough, green to light-green; and the abaxial face is slightly gray. Teak flowers are bisexual, reach 6-8 mm in diameter and are arranged in large panicles. The androecium consists of 6 or 7 stamens that are attached at the corolla tube. The pistil is composed of a double-carpel ovary with 4 ovules. The pollinators are insects, and the flowering duration varies from 2-4 weeks.

Some of these characteristics have been used to differentiate teak phenotypes in provenance trials (Kjaer et al. 1995; Sreekanth et al. 2014), and genetic information has been used to differentiate provenances (Fofana et al. 2009; Verhaegen et al. 2010; Alcântara and Veasey 2013; Sreekanth et al. 2014); however, no characterization descriptors have officially been described for teak.

Because the study of botanical morphological traits is the first step toward establishing characterization descriptors, the aim of this research was to provide fifteen morphological traits for teak that could direct future studies in the establishment of characterization descriptors.

Material and methods

Plant material

Branches with leaves and flowers were collected from farms belonging to the FLORESTECA Company near Cáceres city in the state of Mato Grosso, Brazil (16°4'S, 57°40'W, 165 m above sea level). The Cáceres provenance refers to the branches of different individuals from the seeds of the FLORESTECA Company.

In addition, branches from clones belonging to the PROTECA Company were sampled. These clones were named the Thailand (Maeka and Maehuad), Indonesia (Cepu) and the Solomon Islands provenances.

Seventy eight individuals originated from seeds were collected at Cáceres, eight five samples were collected from Solomon Islands clones, forty nine samples were collected from Indonesia clones and sixty three samples were collected from Thailand clones. The branches were wrapped in newspaper, placed in plastic bags and kept in hydrated alcohol 93.8% (commercial alcohol 96°GL) to be studied at ESA Herbarium (Fig. 1), in the city of Piracicaba, São Paulo State, Brazil.

To facilitate their handling during analysis, the materials were not dried in an oven. After the study was completed, however, some of these materials were dried in an oven to be added to the herbarium as exsiccate.

Figure 1. (A) Green material that was collected from the field (Cáceres - MT, Brazil). (B) Material that was preserved in 93.8% hydrated alcohol for study in laboratory (Piracicaba - SP, Brazil).

Variables studied

We studied fifteen variables of the leaves, inflorescences and flowers in each provenance:

Y_1: leaf petiole length (cm);
Y_2: leaf length (cm);
Y_3: leaf width (cm);
Y_4: inflorescence length (cm);
Y_5: inflorescence width (cm);
Y_6: calyx lobe length (mm);
Y_7: corolla lobe length (mm);
Y_8: fillet length (mm);
Y_9: anther length (mm);
Y_{10}: ovary length (mm);
Y_{11}: number of sepals;
Y_{12}: number of petals;
Y_{13}: number of anthers;
Y_{14}: leaf shape; and
Y_{15}: type of trichome.

The study of trichomes was conducted using Scanning Electron Microscopy (SEM) at the Center of Electron Microscopy Applied to Agricultural Research (NAP/Mepa) at ESALQ/USP. Radford botanical terms (Radford et al. 1974) were adopted for the study of leaf shape.

Analysis of variance

The continuous quantitative variables, such as the measurements of length and width (Y_1 until Y_{10}), were measured using a caliper rule. These data were analyzed using Shapiro-Wilk's test (SAS Institute, 1999), an analysis of variance (ANOVA) and Tukey's test (P≤0.05).

Poisson regression

The non-continuous quantitative variables, such as the numbers of sepals (Y_{11}), petals (Y_{12}) and anthers (Y_{13}), were confirmed by simple counting, and statistical analyses were performed by Poisson regression (Equation 1) using SAS software version 8.2.

$$\log(\mu_i) = \sum_{j=1}^{p} y_{ij}\beta_j \qquad (1)$$

Wherein: μ is the expected value of the variable Y_i (i=11, i=12 and i=13) with Poisson distribution.

Results

Quantitative continuous traits

Leaf

Because the data were normally distributed, Table 1 shows the Tukey's test results. Analysis of the results shows that the average petiole length can differentiate the Solomon Islands, Cáceres and Thailand provenances. On the other hand, leaf length and width can differentiate the Solomon Islands, Indonesia and Thailand provenances.

It is notable that for leaf length and width, the Cáceres provenance differed greatly only from the Thailand provenance.

For petiole length, the Cáceres provenance not only differed from Thailand but was also significantly different from the Solomon Islands. Moreover, for this trait, the Indonesia and Thailand provenances did not differ.

Table 1. Analysis of the averages of the leaf variables for each provenance.

Provenance	N	LPL (cm)	LL (cm)	LW (cm)
Solomon Islands	85	1.5(±0.6)^{a}	20.7(±6.0)^{a}	14.5(±3.5)^{a}
Cáceres	78	2.2(±0.6)^{b}	18.5(±4.5)^{ab}	13.5(±3.5)^{ab}
Indonesia	49	2.1(±0.9)^{bc}	17.9(±6.5)^{b}	12.7(±4.6)^{b}
Thailand	63	1.8(±0.6)^{c}	12.8(±4.0)^{c}	9.2(±3.2)^{c}

In column, means followed by different letters differ significantly by Tukey's test. N - number of samples; LPL - leaf petiole length; LL - leaf length; LW - leaf width. Values presented as: mean (±standard error).

Inflorescence

Table 2 shows the results of the statistical analysis using Tukey's test for the average inflorescence length and width.

Considering the inflorescence length, the Indonesia provenance differed from the Solomon Islands and Cáceres provenances but not from the Thailand provenance. The Solomon Islands, Thailand and Cáceres provenances did not differ.

Tukey's test had different results for the analysis of the average inflorescence width compared to that of the inflorescence length. Although the Indonesia provenance was similar to the Cáceres provenance, it differed greatly from other provenances, presenting the largest inflorescences.

Table 2. Analysis of the average inflorescence variables for each provenance.

Provenance	N	IL (cm)	IW (cm)
Solomon Islands	25	27.6(±2.2)^{a}	25.7(±4.6)^{ab}
Cáceres	24	29.0(±6.3)^{a}	28.2(±5.9)^{ac}
Indonesia	25	32.4(±6.3)^{b}	30.3(±7.3)^{c}
Thailand	37	29.6(±3.8)^{ab}	23.4(±4.5)^{b}

In column, means followed by different letters differ significantly by Tukey's test. N - number of samples; IL - inflorescence length; IW - inflorescence width. Values presented as: mean (±standard error).

Flower

When analyzing the characters of the flowers (Table 3), we noted that their pattern varied between provenances.

The size of the ovaries from Cáceres provenance differed from those of the Solomon Islands and Indonesia. Only the Solomon Islands provenance showed a greater statistical average (differential average) fillet length than that of the other provenances. This type of variation was also observed in the average anther length, calyx lobe length and corolla lobe length, for which the Solomon Islands provenance had larger measurements that were significantly different from those of the other provenances.

Table 3. Analysis of the average length of the variables related to the flowers in each provenance.

Provenance	N	Ovary (mm)	Fillet (mm)	Anther (mm)	Calyx lobes (mm)	Corolla lobes (mm)
Solomon Islands	62	1.26(±0.33)[a]	3.23(±0.86)[a]	2.90(±0.56)[a]	1.61(±0.34)[a]	2.22(±0.41)[a]
Indonesia	67	1.24(±0.28)[a]	2.52(±0.73)[b]	1.93(±0.46)[b]	1.43(±0.29)[b]	1.80(±0.39)[b]
Thailand	31	1.13(±0.36)[ab]	2.71(±0.83)[b]	2.17(±0.48)[b]	1.45(±0.30)[b]	1.99(±0.50)[b]
Cáceres	49	1.04(±0.14)[b]	2.76(±0.72)[b]	2.08(±0.46)[b]	1.48(±0.32)[b]	1.85(±0.43)[b]

In column, means followed by different letters differ significantly by Tukey's test. N - number of samples. Values presented as: mean (±standard error).

Quantitative non-continuous traits

Flower

Quantitative non-continuous traits, such as the number of petals, sepals and anthers, were analyzed using Poisson regression. The most frequent number of petals and sepals was 6, and this frequency did not differ statistically between provenances. The Cáceres and Indonesia provenances differed in the number of anthers (from 6 to 7; Table 4).

Table 4. Analysis of the number of anthers by Poisson regression.

Provenances		*p-value*
Thailand	Cáceres	0.9854
	Indonesia	0.7555
	Solomon Islands	0.9972
Solomon Islands	Cáceres	0.9820
	Indonesia	0.7535
	Thailand	0.9972
Indonesia	Cáceres*	0.0019
	Solomon Islands	0.7535
	Thailand	0.7555
Cáceres	Indonesia*	0.0019
	Solomon Islands	0.9820
	Thailand	0.9854

* Values lower than 0.05 represent significantly different provenances.

Qualitative traits

Trichomes types

Four different types of trichomes were found on abaxial surface (Fig. 2): erect, suberect, arachnoid, glandular and star shaped.

The Cáceres provenance had an abundance of suberect trichomes, similar to a carpet covering the entire leaf (Fig. 2A). In the Indonesia provenance, suberect, erect and glandular trichomes were observed (Fig. 2B). Note that the arachnoid trichomes on the abaxial face of the leaves are unique in the Solomon Islands provenance (Fig. 2C). In the Thailand provenance, suberect, glandular and star-shaped trichomes were found (Fig. 2D).

Leaf shape

Considering the leaf blade shape, all of the provenances had elliptical or oboval leaves (Fig. 3). Moreover, oval leaves were observed in only three provenances: Indonesia, Thailand and Cáceres. This trait may be another important trait that differentiates Solomon Islands from these provenances. The Thailand provenance possessed 21% oval-shaped leaves and 76% elliptical leaves, while the oboval shape was found in only approximately 3% of the samples. The Cáceres provenance possessed 67% elliptical leaves, 30% oboval leaves and 3% oval leaves. The Indonesia provenance possessed all three shapes with minor percentage differences: 17% oval leaves, 34% oboval leaves and 49% elliptical leaves. In addition, 36% of the leaves of the Solomon Islands provenance were oboval and 64% were elliptical.

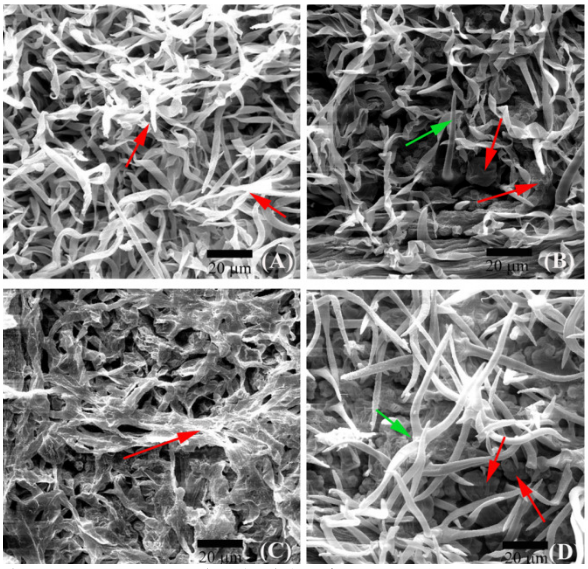

Figure 2. *Tectona grandis*, abaxial surface. (A) Cáceres provenance. Suberect trichomes (red arrows). (B) Indonesia provenance. Erect trichome (green arrow) and glandular trichomes (red arrows). (C) Solomon Islands provenance. Arachnoid-shaped trichome (red arrow). (D) Thailand provenance. Star-shaped trichome (green arrow) and glandular trichomes (red arrows). Images obtained from scanning electron microscopy (200×).

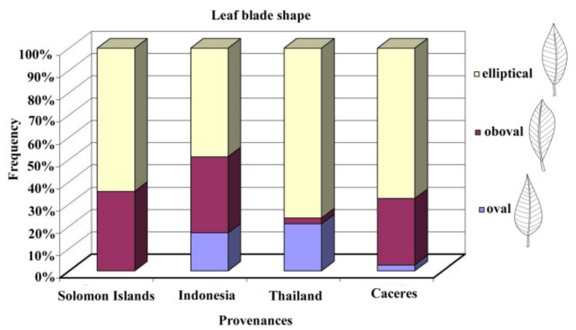

Figure 3. Distribution of leaf blade shape for the Solomon Islands, Indonesia, Thailand and Cáceres provenances.

The shape of the leaf apex was also observed (Fig. 4); however, only the Thailand provenance presented a very low percentage of acuminate apex (only 3% of the leaves analyzed) (Fig. 4A). The frequencies of leaves with an acute apex were 71%, 81% and 78% on Solomon Islands, Indonesia and Cáceres, respectively (Fig. 4B).

Figure 4. Shapes of apex leaves found for teak. (A) Acuminate apex. (B) Acute apex.

Discussion

Considering the quantitative traits that were studied for teak, Cáceres provenance was more similar to Indonesia provenance, possessing ten similar quantitative traits: leaf length, leaf width, petiole length, inflorescence width, fillet and anther lengths, calyx lobe and corolla lobe lengths, and number of sepals and petals (Tables 1, 2 and 3). The Thailand provenance had nine similar characteristics to Cáceres: inflorescence length, ovary length, fillet and anther lengths, calyx lobe and corolla lobe lengths, and number of sepals, petals and anthers (Tables 2, 3 and 4). The Solomon Islands provenance was the least similar to Cáceres, possessing only seven similar characteristics: leaf length, leaf width, inflorescence length, inflorescence width, and number of sepals, petals and anthers (Tables 1, 2 and 4).

The number of sepals and petals are characteristics that are not interesting for use as descriptors because there was no variation among the provenances. Vasudeva et al. (2004) also did not use these parameters to differentiate teak clones; instead, they used the length and diameter of floral constituents.

When qualitative traits for teak are concerned, leaf shape characteristics are not interesting for use as descriptors because the apex type most frequently observed was acute for all provenances. However, the type of trichome is an interesting characteristic because it varies depending on the provenance. The Cáceres provenance possessed suberect trichomes, whereas Indonesia and Thailand possessed both suberect and glandular trichomes. The differences between the latter two provenances were the existence of erect trichomes in Indonesia and the presence of star-shaped trichomes in Thailand. The Solomon Islands provenance was the most diverse among all of the provenances studied, possessing a unique type of trichome (arachnoid). This result agrees with genetic data that were obtained from microsatellite markers by Alcântara and Veasey (2013), who described the Solomon Islands provenance as genetically distant to the Cáceres provenance. These observations indicate that the trichome type has the potential to become a descriptor for this species because this characteristic is highly heritable and follows Mendelian segregation (Van Dam et al. 1999; Aruna et al. 2005); however, additional studies are necessary to verify the hereditability of other characteristics, such as the quantitative traits that were evaluated for teak.

Conclusion

Among the thirteen quantitative variables that were studied, eleven had significant differences: leaf length and width; petiole length; inflorescence length and width; ovary, fillet and anther lengths; calyx lobe and corolla lobe lengths; and the number of anthers. Among the two qualitative traits, the type of trichome could be a useful trait for differentiating teak provenances. These characteristics could be helpful as preliminary information to foster the development of characterization descriptors for genetic breeding programs and for the protection of teak cultigens.

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