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Seed and seedling morphology of *Bauhinia scandens* L.

Morfologia de semente e plântula de *Bauhinia scandens* L.

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Highlights

Morphological description of *B. scandens* aids in its identification in the field.
Majority of high density seeds produce normal seedlings.
Reddish petioles distinguishes *B. scandens* from other species of the same genus.

Abstract

Bauhinia scandens has potential importance as an ornamental and medicinal plant. Researchers have isolated and identified 1-O-alkylglycerol in the leaves of the *B. scandens* plant, and established antitumor properties using the Brine Shrimp toxicity test, an internationally accepted bioassay. Although this species has high potential, little is known about the viability of seedling production and the morphology of these plants, particularly in terms of seed characteristics and initial stages of germination. The objective of this study was to characterize the seed morphology, germination, and seedlings of *B. scandens.* Seed water content, weight, and coloration were evaluated. This study also included a description of seed biometrics, external and internal structures, germination, and seedling morphology. Internal seed morphology was evaluated by the anatomical sectioning and X-ray methods. The morphology data obtained were subjected to descriptive statistical analysis and germination data were determined using Cramér's V. *B. scandens* seeds have a coloration ranging from very dark grayish-red to dark reddish-brown, flat oblong shapes, and rounded bases and apexes with full or slightly undulating margins. Healthy seedlings are produced mainly by seeds with well-formed internal structures. The reddish petiole of the seedling leaves is a taxonomic character for *B. scandens* identification. The non-domestication and genetic variability of this species reflect on the seed and seedling color and size variation.

Key words: Climbing bauhinia. Density. Fabaceae. Internal structure. X-ray.

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Resumo

Bauhinia scandens tem potencial como planta ornamental e importância medicinal. Nas folhas da planta de *B. scandens,* foi relatado pelos pesquisadores, o isolamento e identificação de glicerol 1-O-alquilo, os mesmos estabeleceram a propriedade antitumoral por um bioensaio, aceito internacionalmente denominado teste de toxicidade de Brine Shrimp. Por ser ainda pouco conhecida e com alto potencial exploratório, a viabilidade da produção de mudas e maior conhecimento sobre sua morfologia se faz necessária. Faltam informações sobre as características das sementes e estágios iniciais de germinação. O objetivo deste estudo foi caracterizar o tipo morfológico de sementes, germinação e plântulas de *B. scandens.* Foram avaliados o teor de água, o peso de mil sementes e a coloração das sementes. Este estudo também incluiu a descrição e biometria da semente, estrutura (externa e interna), germinação e morfologia das plântulas. A morfologia interna das sementes foi avaliada por corte anatômico e método de raios-X. Os dados morfológicos obtidos foram submetidos à análise estatística descritiva e os dados de germinação foram testados utilizando Cramér's V. *B. scandens* apresentam coloração que varia de vermelho acinzentado muito escuro a marrom avermelhado escuro e o tipo de semente é de forma oblonga plana, base e ápice são arredondados e cheios ou margens ligeiramente onduladas. Plântulas normais são produzidas majoritariamente por sementes com uma estrutura interna bem formada. O pecíolo avermelhado das folhas da plântula é um caráter taxonômico para identificação de *B. scandens.* A não domesticação e a variabilidade genética desta espécie refletem na variação da cor e tamanho das sementes e plântulas.

Palavras-chave: Bauhinia trepadeira. Densidade. Fabaceae. Estrutura interna. Raios X.

Introduction

Bauhinia scandens L. is originally from Southeast Asia, specifically East India where it is found in tropical and subtropical forests (Bandyopadhyay, Thothathri, & Sharma, 2005) and considered an important ornamental vine and medicinal plant of the Fabaceae family.

Hazra and Chatterjee (2008) verified the antitumor attributes of the 1-O-alkylglycerol compound extracted from the leaves of this species, suggesting promising uses of the plant in herbal medicines, whereas Hossain, Niloy, Hosen and Islam (2016) found the presence of anti-oxidant activity in *B. scandens* extracts that can be useful in food preservation. Poonsri, Pluempanupat, Chitchirachan, Bullangpoti and Koul (2015) identified insecticidal action using leaf extracts for the control of *Plutella xytostella,* one of the main pests found in brassicas.

B. scandens has excellent potential for economic exploitation. However, the morphology of seeds and seedlings, as well as the mechanism of seedling production, remain poorly understood. Knowledge of seed and seedling morphology can aid in such areas as taxonomic studies, laboratory germination test interpretations, nursery work, and species ecology research (Ferreira & Barretto, 2015).

Differences between species, observed mainly in the early stages of growth, included seedling organ coloration, developmental speed, venation, and leaf shape (G. M. C. Silva, Silva, Almeida, Farias, & Lima, 2003; Borges & Mendonça, 2009; Khan, Zaki, & Anis, 2015).

X-ray analysis can be used to aid morphological descriptions (Jeromini, Martins, Pereira, & Gomes, 2019; L. A. Silva, Sales, Santos, Martins, Costa, & Silva, 2017) since it is straight forward, rapid, and accurate in the identification of structures that are imperceptible by conventional techniques (Gomes, 2010).

This method also allows for the observation of seedlings originating from seeds with various malformations, damage, and tissue densities (Leão-Araújo, Gomes, Silva, Peixoto, & Souza, 2019). Thus, the objective of this study was to morphologically characterize the seeds, germination type, and seedlings of *B. scandens.*

Materials and Methods

Sample collection and processing

The fruit of B. scandens was harvested from 10 mother plants in Botucatu, São Paulo, Brazil (22º53'09'' S and 48º26'42'' W) and sent to the Seed Analysis Laboratory of the Department of Plant Production of UNESP, Jaboticabal, São Paulo. The seeds were extracted from the fruit and homogenized. The water content was determined through the oven method at 105 ± 3 °C for 24 h. The results were expressed as a percentage. The weight of 1000 seeds was determined and expressed in grams (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2013).

Biometrics and external morphology of the seed

One hundred seeds were randomly selected and length, width, and thickness were measured using a digital caliper. Results were expressed in millimeters (Dutra, Cardoso, Souza, Bandeira, & Morais, 2016). The seeds were analyzed using a 10× magnification binocular stereo microscope, gauging the structures with criteria established by Borges and Mendonça (2009) and The Rules for Seed Analysis – MAPA (2009).

The following characteristics were described: color, shape, surface type, base type, and apex type, along with aspects of the hilum, micropyle, and raphe. Seed coloration was verified by comparison with the Munsell color catalog (Munsell, 1976) and results expressed in hue (determined by wavelength), value (brightness or light intensity), and chroma (color saturation).

Internal structure of the seed by the anatomical cutting method

The selected seeds were immersed in distilled water for 24 h at 25 °C to soften the tissues and facilitate anatomical cutting, which was performed with a scalpel in the longitudinal direction between the cotyledons and through the embryonic axis. The internal structures were analyzed with the aid of a 10× magnification binocular stereo microscope. The color, form, surface type, cotyledon characteristics, and the embryonic axis were described (Borges & Mendonça, 2009; MAPA, 2009).

Internal structure of seed by the X-ray method

Five hundred seeds on double-sided tape adhered to a 1 mm thick transparent acetate film were used. The seeds were positioned 14.3 cm from the X-ray emission source of the Faxitron X-ray model MX 20

DC 12 machine to obtain digital radiographs (Abud, Cicero, & Gomes, 2018).

Radiographic images were analyzed, compared to those obtained by the anatomical cutting method, and separated into five classes according to their internal characteristics, similarly to Jeromini et al. (2019). Seeds of each class were scarified on the opposite side of the hilum with 220-grade sandpaper to overcome dormancy and maintain individuality.

The seeds were placed between two rolled paper towels moistened with 25 °C water at 2.5× the weight of the paper towels for 26 days for germination. After which, images of the internal structure of seeds generated by X-rays were compared to determine the percentages of formed seedlings or dead seeds (MAPA, 2013; Abud et al., 2018).

Germination and seedling morphology

In order for the seeds to overcome dormancy and germinate, seeds were immersed in 40 mL of 36 N 95% sulfuric acid for 60 min, rinsed continuously with water through a sieve for 10 min until the acid residues were removed, and dried in the shade (25 °C and 50% RH) on paper towels for 24 h (Jeromini, Pereira, Silva, & Martins, 2020).

Sowing was performed with four repetitions of 50 seeds in autoclaved sand, moistened with water at 60% retention capacity (MAPA, 2013), inside plastic boxes (22 × 15 × 5 cm). The boxes were kept in a greenhouse (26 ± 3 °C and 60% RH). Measurements and descriptions of the parts of the seedling were performed on samples of 20 normal seedlings obtained at two developmental stages: before true leaf formation (Phase I) and after true leaf expansion (Phase II) (Leonhardt, Bueno, Calil, Busnello, & Rosa, 2008).

Prior to the formation of true leaves, the length and width of the cotyledonary leaves and the length and diameter of the hypocotyls were measured. After the expansion of the true leaves, the length and width of the first pair of leaves (two open leaflets), and the lengths and diameters of the hypocotyl, epicotyl, and primary root were determined.

The evaluations were made with the aid of a digital caliper and a millimeter ruler. Results were expressed in millimeters (G. M. C. Silva et al., 2003; Leonhardt et al., 2008; Borges & Mendonça, 2009; Dutra et al., 2016).

A photographic register of the entire germination process and seedling emergence was recorded, as well as the identification of morphological aspects of the seedlings with emphasis on the relevant particularities for the recognition of the species in the field or in a controlled environment, from the emergence of the primary root to that of the second true leaf (Leonhardt et al., 2008).

Statistical analyses

Data on seed biometrics and seedling structure dimensions were subjected to descriptive statistical analysis to obtain the mean, maximum and minimum dimensions, standard deviation, and coefficient of variation using AgroStat.

The germination percentage data were tested for structure by status using SAS software (v 9.2, SAS, Inc., Cary, NC, USA). PROC FREQ was used to construct contingency tables and analyze all categorical data. The degree of association between variables was analyzed by computing Cramér's V (Sheskin, 2020). Significance was evaluated at $\alpha = 0.05$.

Results and Discussion

On average, the seeds had 6% water content and 1000 seeds had a weight of 34.6 g. The shape of the seeds was classified as flat oblong, with a rounded base and apex, and full or slightly undulating margins. The average seed was 6.91 mm long, 4.34 mm wide, and 2.29 mm thick, with a standard deviation of 1.52, 0.95, and 0.19, respectively (Table 1).

This standard deviation data reflects a larger difference between seed length and a smaller difference between seed thickness. The size variation can be considered within the normal range for this genus, as it was also observed for *B. monandra* (Borges & Mendonça, 2009) and *B. forficata* (Dutra et al., 2016).

The seed coat of *B. scandens* showed variable coloration between very dark grayish red; 2.5 YR 2.5/2, and dark reddish-brown; 5 YR 2.5/2 (Munsell, 1976). The surface of the *B. scandens* seed is smooth, polished, and slightly convex (Figure 1A and B).

Table 1
Biometrics of *B. scandens* seeds

	Length	Width	Thickness
	----------[mm]----------		
Maximum	9.52	5.97	2.60
Minimum	3.37	2.12	1.67
Average	6.91	4.34	2.29
Standard Deviation	1.52	0.95	0.19
CV (%)	22.04	21.99	8.41
N=100			

CV- Coefficient of variation.

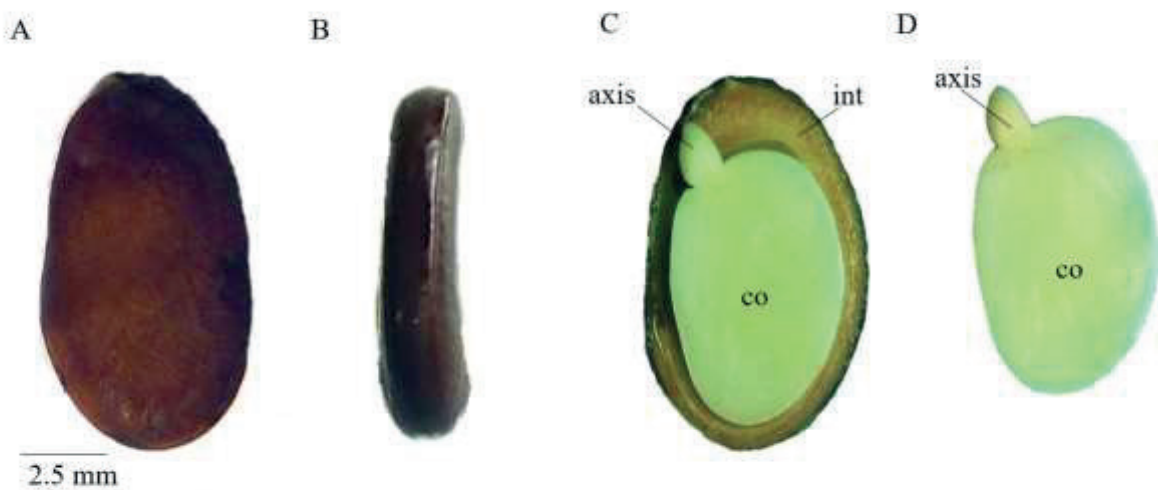

Figure 1. External morphology of the face (A) and lateral side (B) of *B. scandens* seeds and internal structure by anatomical section (C) with visualization of the integument (int), embryonic axis (axis), cotyledon (co) and embryo (D).

The seed had a small circular micropyle and a V-shaped hilum in the apical region. According to Gunn (1981), this V-shaped hilum is a unique feature of the *Bauhinia* genus, and external features such as this enabled the clear identification of family, subfamily, and gender of B. monandra (Borges & Mendonça, 2009).

The hilum was slightly conspicuous and had a very dark grayish red color: 2.5 YR 2.5/2 (Munsell, 1976). The pleurogram, dividing line, micropyle, and lens were absent, and raphe was not visualized in the seeds. The evaluated sample consisted of a mixture of different seed sizes and colors, because undomesticated plants have uneven seed maturation in the pod and high genetic variability among individuals.

Thus, at the time of harvesting, the plot contained heterogeneous seeds in terms of color, size, and density (Mendonça, Martins, Martins, & Lopes, 2015). For other species of the same family such as *Plathymenia foliolosa* Benth (Fonseca, Freitas, Mendonça, Souza, & Abdalla, 2013) and *Caesalpinia echinata* Lam. (Ferreira & Barretto, 2015) seed color variability due to different maturity stages was also reported.

The seed embryo was axial, spatulate, invaginated, and dominant (Figure 1C and D). The embryonic axis (hypocotyl radicle) had a rudimentary plumule and radicle, with a slight asymmetry and curvature. The two cotyledons were foliate, flat, thick, and spatulate. Cartilaginous consistency was identified with yellowish-white coloration partially covering the embryonic axis, along with a rounded base, entire margin, and blades close to the embryonic axis only.

Based on X-ray analysis, the internal structure of seeds was separated into the following categories (Figure 2): well-formed, with the inner cavity fully filled with high density reserve tissue, characterized by the clear coloration of the cotyledon (Figure 2A); malformed, with the inner cavity filled with low density reserve tissue, identified by the darkened area in the center of the cotyledon (Figure 2B); deteriorated embryo, totally or partially dark, due to low tissue density (Figure 2C); stunted embryo, partially adhered to the external tissues of the seed, so that a dark area of internal cavity in the seed persists (Figure 2D); or cracked tissues (Figure 2E). The percentage of seeds in each category in the sample is presented in Figure 3A.

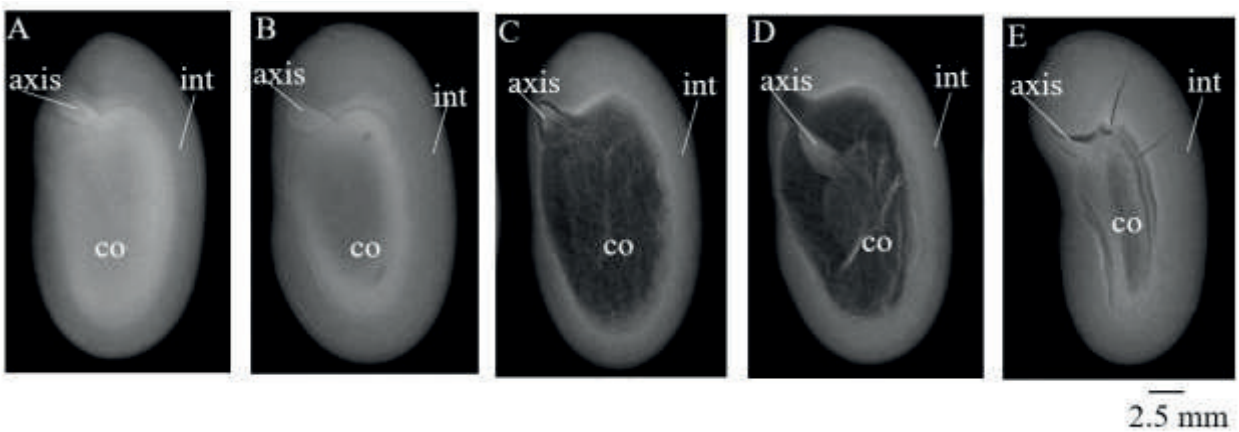

Figure 2. Radiographic images of *B. scandens* seeds categorized as well-formed (A), malformed (B), deteriorated embryo (C), atrophied embryo (D) and cracked (E). axis = embryonic axis; co = cotyledon; int = integument.

X-ray was used to determine the difference between seed tissue densities, with denser and well-formed seeds resulting in brighter images, whereas orifices, damage, or differences in the internal tissues of seeds were identified by a color change and darkened spots (International Seed Testing Association [ISTA], 2009).

Well-formed seeds reflect germination potential and the ability to produce vigorous and normal seedlings. Cramér's V test detected strong levels of association between internal seed morphology and germination capacity (V=0.89) (Table 2). The germination frequency of well-formed seeds was 91.96%, malformed was 8.84%, and the others seed structures did not produce normal seedlings (Figure 3B).

The results from the germination test are shown in Figure 4. The majority of the well-formed seeds gave rise to normal seedlings, as did some of the malformed seeds (Figure 4A), whereas most of the malformed seeds and the remaining samples resulted in dead seeds, which displayed microorganisms on their surfaces at the end of the test (Figure 4B, C, D, and E).

The clearest images observed in Figure 4A reveal the excellent seed and the accumulation of reserve substances. The high density is reflected in germination potential and the ability to produce a vigorous and normal seedling. Similar results were found for the seeds of *Brachiaria brizantha* Hochst. Rich (Jeromini et al., 2019), *Campomanesia adamantium* Camb. (Leão-Araújo et al., 2019), and Brassica oleraceae L. (Abud et al., 2018).

Table 2
Statistics of structure by status of germination of well-formed, malformed, deteriorated embryo, atrophied embryo, and cracked *B. scandens* seeds evaluated by the X-ray method

Descriptive analysis	DF	Value	Probability
Chi-Square	4	404.4272	<.0001
Likelihood Ratio Chi-Square	4	405.0432	<.0001
Mantel-Haenszel Chi-Square	1	214.3793	<.0001
Phi Coefficient		0.8994	
Contingency Coefficient		0.6687	
Cramer's V		0.8994	

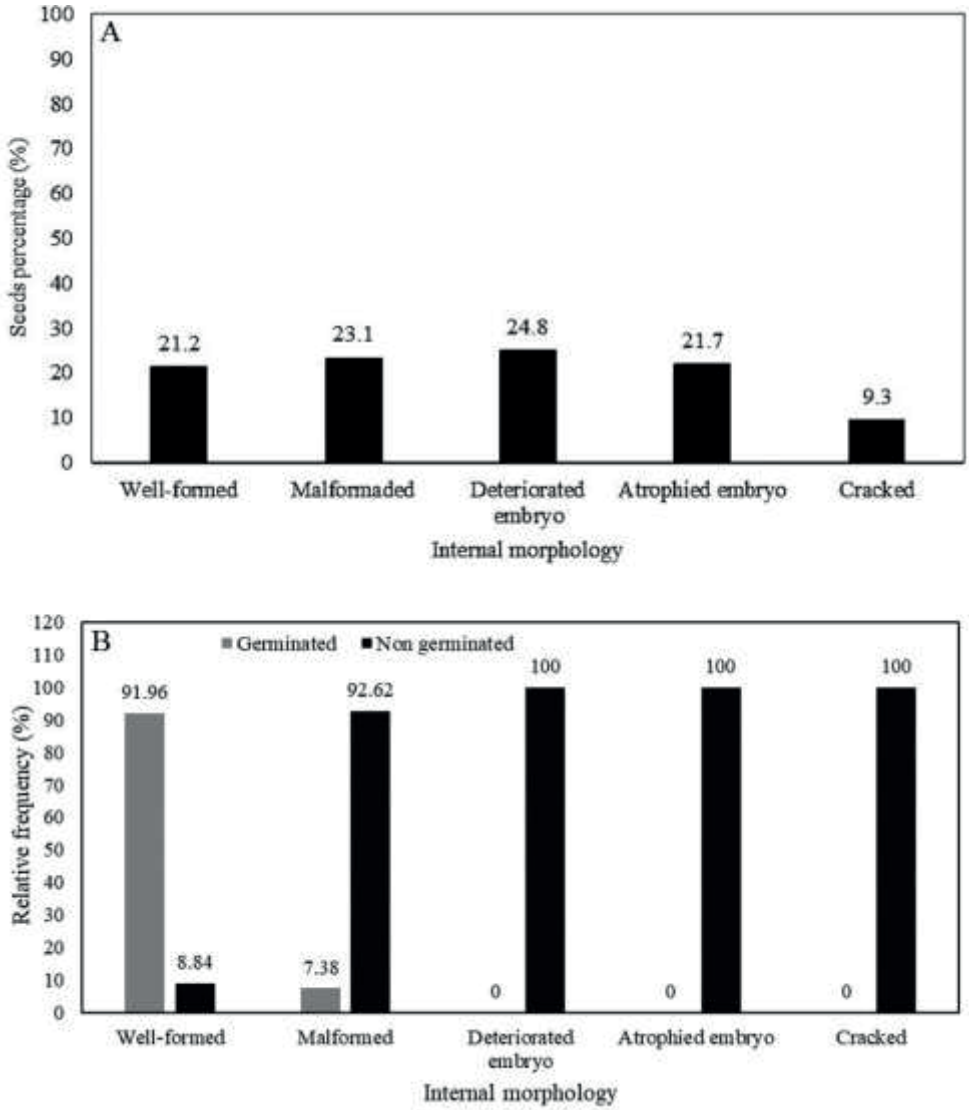

Figure 3. Percentage of seeds (A) and relative frequency of germination (B) of well-formed, malformed, deteriorated embryo, atrophied embryo, and cracked *B. scandens* seeds by X-ray.

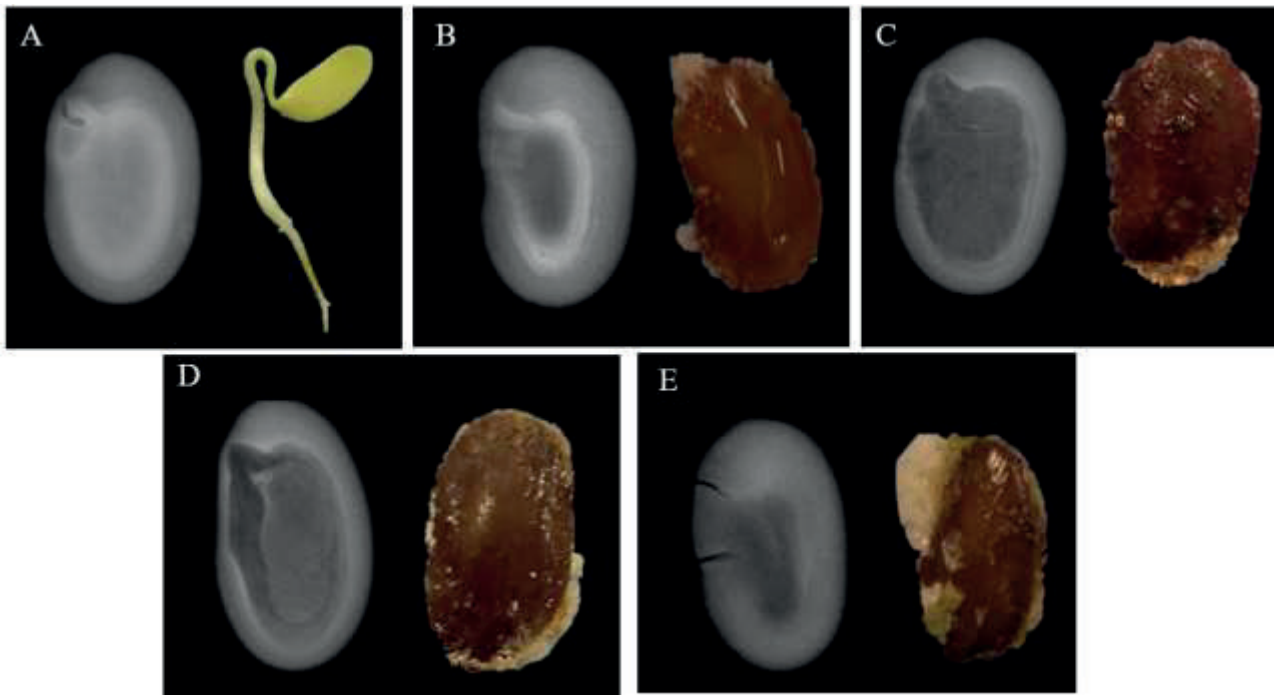

Figure 4. Images obtained in the middle of the germination test by X-ray (left) and at the end of the germination test by binocular stereo microscope (right) show *B. scandens* seeds well-formed (A), malformed (B), with deteriorated embryo (C), with atrophied embryo (D), and cracked (E).

Therefore, the X-ray test can be used to select well-formed seeds associated with high physiological quality expression for seedling production (Severino, Lima, & Beltrão, 2006). This is a non-destructive method and with low radiation doses in low-density tissues, such as seeds, it does not cause mutations in the cell nucleus (ISTA, 2009).

The germination of *B. scandens* seeds was epigeal and occurred with the development of the embryonic axis between the cotyledon and phanerocotyledon seedling formation, beginning with the rupture of the integument by the primary root in the micropyle area. Seedlings began emerging from the 6th to the 8th day after sowing, and seedlings started to develop on the 9th day after sowing, from the sharp stretching of the hypocotyl and the cotyledons above the ground.

The dimensions of the main structures of the seedling are presented in Table 3: hypocotyl, epicotyl, and cotyledons (Phase I); and first true leaf, shoot, and root (Phase II). In Phase I on the 10th day after sowing, the hypocotyl was 6.69 mm in length and 1.36 mm in diameter, both with a standard deviation of 0.059, and the hypocotyl diameter was decreased toward the primary root (Figure 5).

From the 11th day after sowing, the root system displayed a branched, light brown, pivoting root, and the secondary roots were shorter and smaller in diameter than the main root.

The hypocotyl was described is glabrous, with a green color in the region near the cotyledons, becoming gradually whitish in the portions approaching the ground on the 11th day after sowing. Cotyledons were identified as glabrous, semi-fleshy, and oblong, with rounded bases and apexes, full margins, and actinodrome venation, which were green, as photosynthesizers, and yellowish in the region near the base. In Phase I, cotyledons averaged 12.07 mm in length and 6.24 mm in width, with standard deviations of 0.81 and 0.61, respectively.

Table 3
Dimensions of the parts of *B. scandens* seedlings

Descriptive analysis	Phase I [mm]					
	Hipocotyl		Epicotyl		Cotyledon	
	L	D	L	D	L	W
Maximum	6.79	1.55	17.51	1.42	13.85	7.43
Minimum	6.60	1.36	15.36	0.88	10.82	5.18
Average	6.69	1.45	16.43	1.12	12.07	6.24
Standard Deviation	0.059	0.059	0.46	0.15	0.81	0.61
CV (%)	1.01	4.06	2.84	13.37	6.75	9.87
N=20						

Descriptive analysis	Phase I [mm]							
	1st True Leaf				Shoot of Plant		Root	
	L	W	PL	PD	L	D	L	D
Maximum	16.95	39.75	9.62	0,49	35.18	1.36	51.47	1.55
Mínimum	10.30	8.10	8.24	0,35	30.87	0.83	35.06	0.54
Average	13.58	16.54	9.04	0,41	33.45	1.12	43.92	1.05
Standard Deviation	1.68	4.53	0.47	0,04	1.55	0.14	5.41	0.40
CV (%)	12.42	27.43	5.29	10,41	4.63	12.91	12.32	38.53
N=20								

CV = coefficient of variation; L = length; D = diameter; W = width; PL = petiole length, PD = petiole diameter.

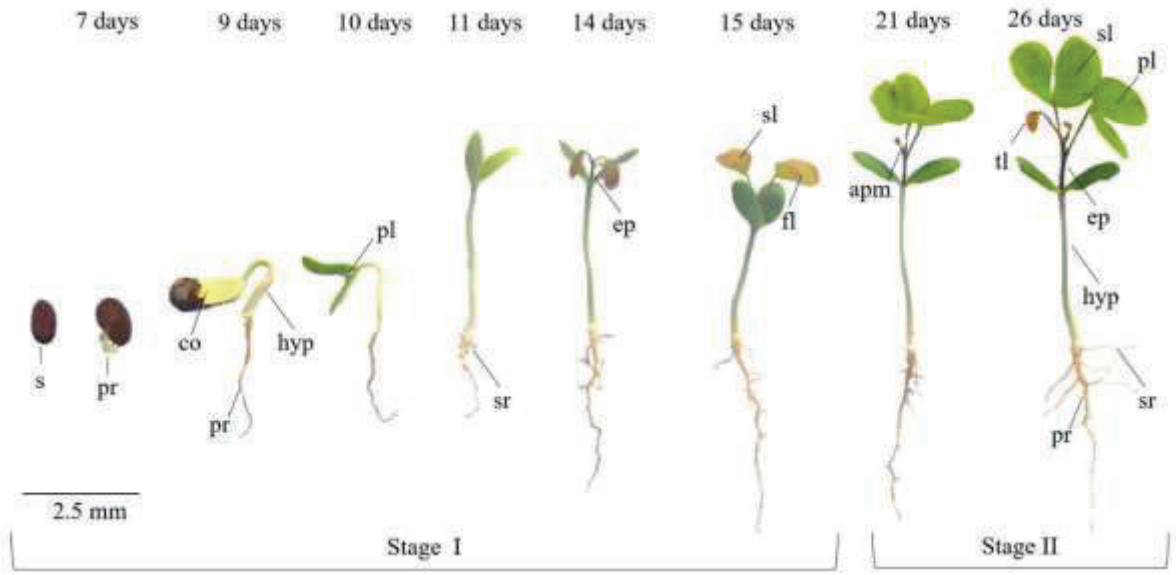

Figure 5. Germination to seedling formation in *B. scandens* seeds. Stage I = germination stages before the expansion of the first true leaf; Stage II = germination stages after the expansion of the first true leaf; s=seed, co=cotyledon, hip=hypocotyl, rp=primary root, sdl=seedling, sr=secondary root, ep=epicotyl, pl=primary leaf, sl=secondary leaf, apm=apical meristems, tl= third leaf.

On the 12th day after sowing, epicotyls started to grow. They were short and reddish-brown, cylindrical, and somewhat hairy. The epicotyl had a mean length of 16.43 mm and mean diameter of 1.12 mm, with standard deviations of 0.46 and 0.15, respectively.

The formation of the first true leaf was observed between the 13th and 14th day after sowing, with the gradual development of the epicotyls and formation of new leaves (Figure 5). The *B. scandens* seedling has conduplicate prefoliation, minimal hair, and a reddish color when young, becoming green as it develops. Near the 18th day after sowing, the second leaf formation began, prior to the full development of the first true leaf.

Phase II began on the 21st day after sowing, at which time the first expanded true leaf was observed. The leaf was classified as membranous, and bilobed with a rounded base, entire margins, actinodrome venation, and three veins in each leaflet.

These characters were also described for *B. forficata* and *B. variegata* (Lusa & Bona, 2009). The true leaves had two leaflets next to the petiole, united only by the base, and the leaflets were glabrous in the adaxial face and hairy in the abaxial face.

Therefore, variability among *B. scandens* seedlings was verified in the size of the first leaf. The species *B.malabarica* and *B. rufescens* had longer Phase II leaf lengths than those observed in *B. scandens,* 17.00 vs 35.00 mm, respectively (Seetharam & Kotresha, 1998).

Between the 21st and 23rd day after sowing, the first true leaf had means of 13.58 mm in length and 16.54 mm in width, with standard deviations of 1.68 and 4.53, respectively (Table 3). Long, cylindrical, hairy,

and reddish petioles were found at all stages of leaf development for the true leaves of *B. scandens* (Figure 5).

The petiole color found here had not been described in the literature for other species of the same genus and can thereby be used as a distinguishing characteristic. The petioles of these leaves had means of 9.04 mm in length and 0.41 mm in diameter, with standard deviations of 0.47 and 0.04, respectively (Table 3).

Two straight, greenish stipules were identified at the base of the petiole, along with a globose, green extrafloral nectary between the two stipules for each leaf, differing from other species such as *B. curvula* which possesses a pair of floral nectaries (Rezende, Cardoso, & Vannucci, 1994).

On the 26th day after sowing, the seedlings presented two expanded leaves with a third beginning to develop. From the development of the second and third leaves, alternate phyllotaxy was identified.

The aerial part of the seedling, from the insertion of the first leaf to the stem at ground level, had a length of 33.45 mm and a diameter of 1.12 mm, with standard deviations of 1.55 and 0.14, respectively. The same root diameter was observed in the ground level region of the stem. The pivoting root was 43.92 mm in length and 1.05 mm in diameter with standard deviations of 5.41 and 0.40, respectively (Table 3).

B. scandens seedlings showed developmental speed similar to *B. monandra* (Borges & Mendonça, 2009), with 26 days to final seedling development; however, development exceeded that of *B. microstachya,* whose seedlings required more than two months after sowing to reach the same stage of development (Leonhardt et al., 2008).

Conclusions

In conclusion, *Bauhinia scandens* seeds have coloration ranging from very dark grayish-red to dark reddish-brown and flat oblong shapes with a rounded bases and apexes, and full or slightly undulating margins.

Healthy seedlings are produced mainly by seeds with well-formed internal structures.

The reddish petiole of the seedling leaves is a taxonomic character for *B. scandens* identification.

The non-domestication and genetic variability of this species reflect on the seed and seedling color and size variation.

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