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REVIEW

Validation of quality tests for forest seed species

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Abstract

The Brazilian literature on seed germination testing of tropical forest species, although still insufficient, is extensive for species with economical potential. However, this information is still fragmented and often reported in scientific communications of modest circulation. In this summary, we present the results of a validation process for germination tests on Brazilian forest species, which had as a starting point the ample reviewing of current methods, besides exhaustive pre-testing in certified laboratories. Specific protocols were created for each species, including substrate, temperature, seed disinfection, breaking of dormancy, seedling classification and other information. These protocols were sent to research laboratories and accredited by the Ministry of Agriculture, together with seed lots of three distinct qualities. The presuppositions of normality of the residuals and homoscedasticity of the variations were previously tested and the completely randomized factorial design statistical model was applied to the normal seedlings of each species. After this analysis, germination test methods for seeds of *Astronium fraxinifolium*, *Ceiba speciosa*, *Cybistax antisyphilitica*, *Enterolobium contortisiliquum*, *Guazuma ulmifolia*, *Lafoensia pacari*, *Mimosa caesalpiniaefolia*, *Peltophorum dubium*, *Pseudobombax tomentosum* and *Pterogyne nitens,* all forest species of different Brazilian biomes, were considered to be validated.

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Methodology validation processes for seeds of Brazilian forest species require technological and scientific knowledge. However, the fragmentation of the information on the same species and the very basic reporting of data, often only giving the number of germinated seeds, make the official conversion of methods into rules difficult. The inclusion of forest species in official analysis rules has always been outdated in relation to cultivated species, as reported by Rogers (1967) in a brief history of the inclusion of shrub and tree species in the official American, Canadian and European rules.

The rules for analysis of Brazilian seeds (Ministério da Agricultura e Reforma Agrária, 2009) cover a small number of forest species which do not include native Brazilian species. Part of this scenario is due to the fact that the marginal usage of 'forest seeds' did not put any pressure on commercialization and aspects related to the quality of the seeds. National and international issues about environmental impact have stimulated the demand for seeds of forest species, as have the sectors that commercialize this product and demand guarantees related to quality and identity.

In order to make the inclusion of germination test methodologies for Brazilian forest species viable, the Ministry of Agriculture (MAPA), in a partnership with the National Council for Scientific and Technological Development (CNPq/ MAPA/ SDA process: 578207/2008-7) and supported by the Research Foundation of the state of Minas Gerais (FAPEMIG Process: APQ-02 844/09), recommended the execution

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of two complementary projects developed by the Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil. The validation of methodologies for germination tests on up to 100 Brazilian forest species was proposed, including information related to seed disinfection, methods for overcoming dormancy and evaluation of criteria for normal and abnormal seedlings, supplemented with images and count days.

Species of different Brazilian biomes were chosen in the first group (Caatinga, Savanna, Atlantic Forest and Wetlands; Fig. 1). At least three lots of seeds of distinct physiological quality were collected from each of the species (*Astronium fraxinifolium*, *Ceiba speciosa*, *Cybistax antisyphilitica*, *Enterolobium contortisiliquum*, *Guazuma ulmifolia*, *Lafoensia pacari*, *Mimosa caesalpiniaefolia*, *Peltophorum dubium*, *Pseudobombax tomentosum* and *Pterogyne nitens*). The differences in quality were



Figure 1. Seeds of *Astronium fraxinifolium* Schott ex Spreng (A), *Ceiba speciosa* (B), *Cybistax antisyphilitica* (Mart.) Mart. (C), *Enterolobium contortisiliquum* (Vell.) Morong (D), *Guazuma ulmifolia* Lam. (E), *Lafoensia pacari* A. St.-Hil. (F), *Mimosa caesalpiniaefolia* Benth. (G), *Peltophorum dubium* (Spreng.) Taub. (H), *Pseudobombax tomentosum* (C. Martius & Zuccanini) Robyns (I) and *Pterogyne nitens* Tul. (J). Scale: background grid, 0.5 cm × 0.5 cm. (A colour version of this figure can be found online at http://journals.cambridge.org/ssr).

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Table 1. Methodologies for germination test, including substrate type, temperature, pretreatment, seed disinfection and count days

Species (Family) RNC		Methodology	
Astronium fraxinifolium Schott ex Spreng (Anacardiaceae) 23 512	Substrate	Rolled paper towel	
	Temperature/light regime	25°C/continuous light	
	Pretreatment	–	
	Disinfection	Detergent solution	
	Counting	1st counting	7 d
		2nd counting	10 d
Ceiba speciosa (A. St.-Hil.) Ravenna (Malvaceae) 23 717	Substrate	Rolled paper towel	
	Temperature/light regime	25°C/continuous light	
	Pretreatment	–	
	Disinfection	Commercial solution of sodium hypochlorite	
	Counting	1st counting	7 d
		2nd counting	10 d
Cybistax antisyphilitica (Mart.) (Bignoniaceae) 23 668	Substrate	Rolled paper towel	
	Temperature/light regime	25°C/continuous light	
	Pretreatment	–	
	Disinfection	Detergent solution	
	Counting	1st counting	14 d
		2nd counting	35 d
Enterolobium contortisiliquum (Vell.) Morong (Fabaceae) 24 025	Substrate	Rolled paper towel	
	Temperature/light regime	25°C/continuous light	
	Pretreatment	Manual scarification	
	Disinfection	Detergent solution	
	Counting	1st counting	7 d
		2nd counting	14 d
Guazuma ulmifolia Lam. (Malvaceae) 24 131	Substrate	Filter paper/container	
	Temperature/light regime	25°C/continuous light	
	Pretreatment	Moist-heat treatment at 90°C + 1h imbibition	
	Disinfection	Detergent solution	
	Counting	1st counting	10 d
		2nd counting	14 d
Lafoensia pacari A. St.-Hil. (Lythraceae) 24 305	Substrate	Rolled paper towel	
	Temperature/light regime	25°C/continuous light	
	Pretreatment	–	
	Disinfection	Detergent solution	
	Counting	1st counting	14 d
		2nd counting	21 d
Mimosa caesalpiniaefolia Benth. (Fabacaeae) 12 505	Substrate	Rolled paper towel	
	Temperature/light regime	25°C/continuous light	
	Pretreatment	Cutting the seed coat	
	Disinfection	Detergent solution	
	Counting	1st counting	21 d
		2nd counting	28 d
Peltophorum dubium (Spreng.) Taub. (Fabaceae) 23 304	Substrate	Rolled paper towel	
	Temperature/light regime	25°C/continuous light	
	Pretreatment	Cutting the seed coat	
	Disinfection	Detergent solution	
	Counting	1st counting	7 d
		2nd counting	14 d

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Table 1. *Continued*

Species (Family) RNC		Methodology	
Pseudobombax tomentosum (Mart. & Zucc.) A. Robyns (Fabaceae) 25 344	Substrate	Rolled paper towel	
	Temperature/light regime	25°C/continuous light	
	Pretreatment	–	
	Disinfection	Commercial solution of sodium hypochlorite	
	Counting	1st counting	10 d
		2nd counting	17 d
Pterogyne nitens Tul. (Fabaceae) 25 362	Substrate	Rolled paper towel	
	Temperature/light regime	25°C/continuous light	
	Pretreatment	Cutting the seed coat	
	Disinfection	Detergent solution + commercial solution of sodium hypochlorite	
	Counting	1st counting	7 d
		2nd counting	14 d

RNC, National Registry of Cultivars.

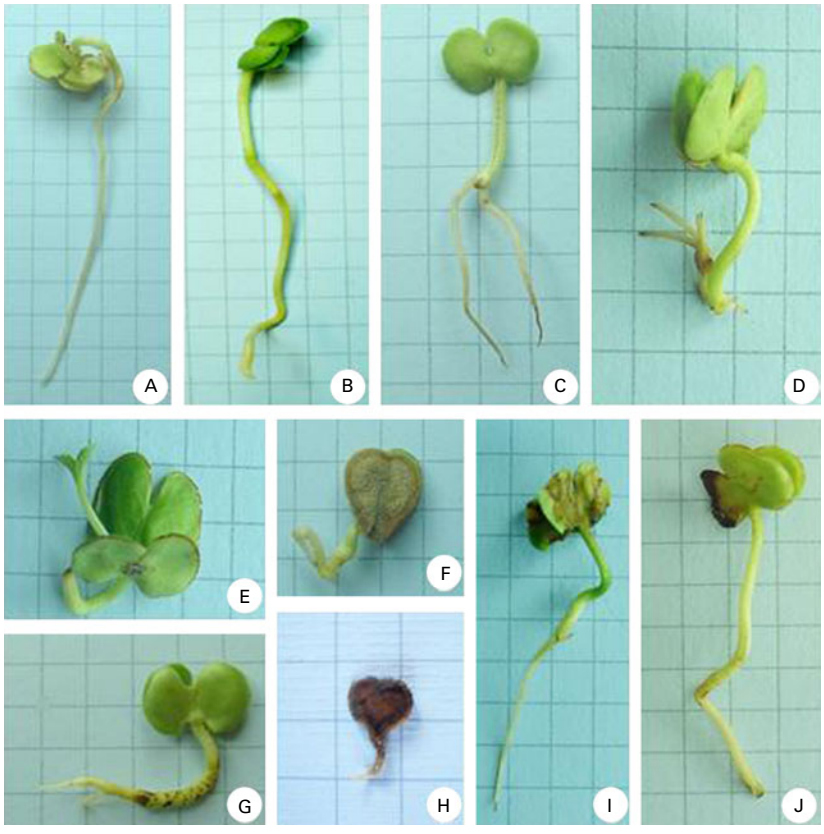


Figure 2. Abnormalities in seedlings of *Cybistax antisyphilitica* (Mart.) Mart.: (A) bent and pigmented hypocotyl; (B) main root with thickening on the extremity; (C) atrophied main root, fungal contamination of the collar region and the extremities of secondary roots, and pigmented hypocotyl; (D) atrophied main root and fungal contamination of the collar region and the extremities of secondary roots; (E) atrophied main root with a necrotic extremity, absence of secondary roots and contaminated cotyledon insertion region; (F) curved and thickened main root and absence of secondary roots; (G) underdeveloped main root and thickened and pigmented hypocotyl; (H) primary infection; (I) main root with contaminated extremity, bending in the collar region and infection present in over 50% of the cotyledons; (J) contaminated main root, absence of secondary roots and pigmented hypocotyl. Scale: background grid, 0.5 cm × 0.5 cm. (A colour version of this figure can be found online at http://journals.cambridge.org/ssr).

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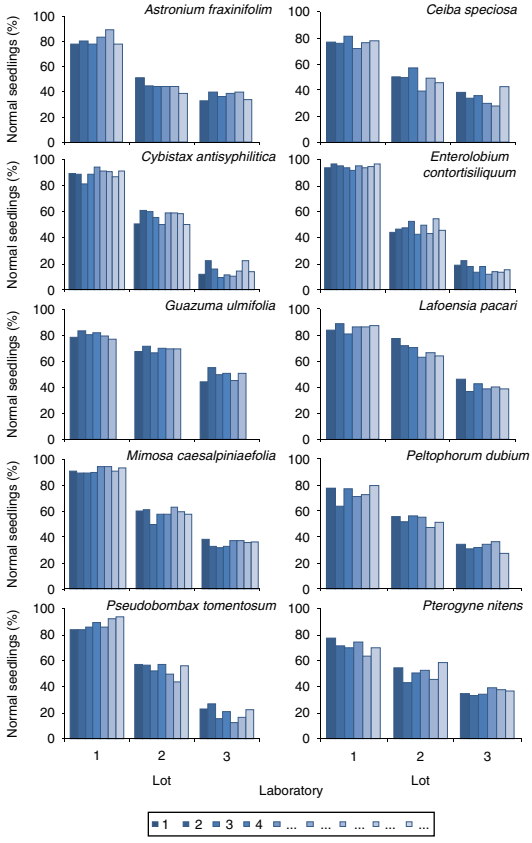


Figure 3. Mean percentage of normal seedlings per lot and laboratory, obtained in the implementation of a validation methodology for testing the quality of seeds of *Astronium fraxinifolium*, *Ceiba speciosa*, *Cybistax antisyphilitica*, *Enterolobium contortisiliquum*, *Guazuma ulmifolia*, *Lafoensia pacari*, *Mimosa caesalpiniaefolia*, *Peltophorum dubium*, *Pseudobombax tomentosum* and *Pterogyne nitens*. (A colour version of this figure can be found online at http://journals.cambridge.org/ssr).

the result of differences in origin, collection year, and degree of fruit and seed maturity, among other things. Seeds from each of the lots were tested and retested after ample bibliographic research of the substrates, germination temperature, pretreatments and parameters that would be most suitable for the evaluation of the quality of the seeds of the species (Table 1).

Seeds from each of the lots were sent to at least six research laboratories, officially recognized by MAPA, and designated as executors, so that the proposed methodology for each species could be tested. The methodology validation germination tests were carried out by researchers and technicians from laboratories at Official Agricultural Laboratory of the state of Goiás (LASO/LANAGRO/GO), Mato Grosso (LASO/INDEA/MT), Minas Gerais (LASO/LANAGRO/MG), Pará (LASO/LANAGRO/PA) Paraná (LASO/CLASPAR/PR), Pernambuco (LASO/LANAGRO/PE), Rio Grande do Sul (LASO/LANAGRO/RS) and São Paulo (LASO/CATI/SP); Brazilian Agricultural Research Corporation (EMBRAPA)/Maize & Sorghum (CNPMS/MG), Genetic Resources & Biotechnology (CENARGEN/DF) and Temperate Agriculture (CPACT/RS); Agricultural Research Foundation of the state of Rio Grande do Sul (FEPAGRO/RS); Laboratory of Seed Analysis of Federal University of Lavras, Minas Gerais (LAS/UFLA/MG); Laboratory of Seed Analysis of Ijuí, Rio Grande do Sul (LAS/UNIJUI/RS); Forestry Science and Research Institute of São Paulo, São Paulo (IPEF/SP).

The moisture content and the 1000 seed weight (variation coefficient below 4%) were determined before shipment of the lots. The laboratories participating in the project received a general protocol

Table 2. Summary of the analysis of variance including the assumptions of the model of the validation process of methods for germination tests of native Brazilian forest species

	MS[1]			Statistics	
Species	Laboratory	Lot	LAB × LOT	*W*	*F*
Astronium fraxinifolium	77.747^{ns}	13,546.097**	50.681^{ns}	0.983	1.393
Ceiba speciosa	135.951^{ns}	10,952.886**	52.423^{ns}	0.987	1.255
Cybistax antisyphilitica	$31{,}388^{ns}$	22,064.802**	50.950^{ns}	0.986	1.820
Enterolobium contortisiliquum	19.503^{ns}	52,845.094**	43.399^{ns}	0.983	1.685
Guazuma ulmifolia	46.113^{ns}	5788.021**	16.950^{ns}	0.980	1.885
Lafoensia pacari	20.633^{ns}	4995.560**	30.738^{ns}	0.977	2.388
Mimosa caesalpiniaefolia	22.211^{ns}	11,091.465**	10.853^{ns}	0.990	1.929
Peltophorum dubium	47.177^{ns}	10,345.764**	58.948^{ns}	0.961	1.149
Pseudobombax tomentosum	120.389^{ns}	27,580.681**	87.881^{ns}	0.969	1.317
Pterogyne nitens	87.413^{ns}	7532.157**	63.359^{ns}	0.975	0.566

[1] MS, mean square; *W*, *F*: statistics for the Shapiro–Wilk test for residual normality and the Levene test for variance homogeneity; ns, no significant difference; $^{**}P < 0.01$ for Snedecor's *F* test.

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with instructions about the execution of the tests and, together with the seeds, specific protocols for the particular species to be analysed, including the germination test methodology, randomization of the plots, seedling images (Fig. 2) and evaluation (see supplementary Table S1, available online only at http://journals.cambridge.org).

After the execution of the protocols, the laboratories sent the analysis cards with the collected data so that they could be analysed graphically (Fig. 3) and statistically (Table 2). The statistical analysis of the data was based on ISO 5725 (accuracy [trueness and precision] of measurement methods and results) and on the validation manual of the International Seed Testing Association (ISTA, 2007). The assumptions of normality of the residuals of the model and variation homogeneity were initially tested, for each species, by the Shapiro–Wilk and Levene tests, respectively. An exploratory analysis eliminated discrepant values (box–plot test) and the variation analysis model was applied. From these projects, official analytical methods were established for germination tests for the seeds of *Astronium fraxinifolium*, *Ceiba speciosa*, *Cybistax antisyphilitica*, *Enterolobium contortisiliquum*, *Guazuma ulmifolia*, *Lafoensia pacari*, *Mimosa caesalpiniaefolia*, *Peltophorum dubium*, *Pseudobombax tomentosum* and *Pterogyne nitens* (Normative instruction No. 44 published in the MAPA official diary on 23 December 2010, http://www.in.gov.br/imprensa/visualiza/index.jsp?jornal=1&pagina=2&data=24/12/2010).

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