Coefficient of variation of normal seedlings obtained from the validation of methods for the seed germination testing of 20 species belonging to the family Fabaceae[1]

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ABSTRACT - The standardization of inter-laboratory results of germination test of forest species seeds requires that the methods be robust. Therefore, the objective was to compare and discuss, through the coefficient of variation for normal seedlings, the variabilities present in the process of validation methods obtained in the germination test for seeds of 20 species of the family Fabaceae. Coefficients of variation for the experiment by lot and by laboratory were calculated for normal seedlings from the statistical analysis of method validations. For normal seedlings of 20 Brazilian forest species, the coefficients of variation are low (up to 9.84%), to average (up to 17.66%), contrary to expectations due to high genetic variability in these barely improved species. The increase of the coefficient is not related to treatment for breaking dormancy, but it grows as the lot quality decreases. The high coefficients by laboratory, overestimated by the lot effect, are uniform indicating that the methods are repeatable. The coefficient is not an indicator capable of predicting the heterogeneity of model variance. As normal distribution models random events, randomness is present in the validation process of the 20 forest species of the Fabaceae family.

Index terms: dormancy, statistical assumptions, forest seeds, data transformation, experimental variability.

Coeficientes de variação de plântulas normais obtidas na validação de métodos para teste de germinação de sementes de 20 espécies florestais da família Fabaceae

RESUMO - A uniformização dos resultados inter laboratoriais de testes de germinação de sementes de espécies florestais exige que os métodos sejam robustos. Assim, o objetivo foi comparar e discutir, por meio do coeficiente de variação obtido para plântulas normais, as variabilidades presentes no processo de validação de métodos obtidas no teste de germinação de sementes de 20 espécies da família Fabaceae. Coeficientes de variação para o experimento, por lote e por laboratório para a variável plântulas normais foram calculados. Os coeficientes de variação obtidos para plântulas normais de 20 espécies florestais nativas, são de baixos (até 9,84%) a médios (até 17,66%), contrariando o esperado pela grande variabilidade genética dessas espécies pouco melhoradas. O aumento do coeficiente de variação não está relacionado ao tratamento utilizado para superação de dormência, porém cresce à medida que a qualidade do lote decresce. Os altos coeficientes obtidos por laboratório, superestimados pelo efeito de lotes, são uniformes indicando que os métodos são reproduzíveis. O coeficiente de variação não é um indício capaz de predizer a heterogeneidade das variâncias do modelo. Como a distribuição normal modela eventos aleatórios, a aleatoriedade está presente no processo de validação de métodos das 20 espécies florestais da família Fabaceae.

Termos para indexação: dormência, pressuposições estatísticas, sementes florestais, transformação de dados, variabilidade experimental.

Introduction

Native species seed trading is still new compared to cultivated seed trading, due to the lack of supervision and the informality of the sector, thus, producers end up using seeds that they have collected themselves (Gonzáles and Torres, 2003). From 2001, the production of native seedlings boosted from the mandatory restoration of areas on farms (Lorza, 2009).

With a new perspective of plant resources, particularly in regard to forestry, Law No. 10.711/2003 (Brasil, 2004) has established a new phase and outlook for the sector of production and marketing of forest seeds and seedlings (Iede, 2005). Among the prospects, germination test standardization stands out.

Germination involves a sequence of biochemical,

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morphological and physiological events (Nonagaki et al., 2010), simplified in initial processes, such as seed imbibition, activation of metabolism (biochemical method), and ending in root protrusion (Labouriau, 1983 ; Nonagaki et al., 2010). The quantification and characterization of germination by seed technologists are determined by the development of embryo structures (Brasil, 2009; Nonagaki et al., 2010; ISTA, 2011).

There are several contributions to the standardization of laboratory procedures for analysis of forest seeds (Wielewicki et al., 2006; Brüning et al., 2011), however, with no assessment of replicability. The interest in standardization arises from the need to accurately determine the quality of seeds (Wielewicki et al., 2006). However, the fragmentation of information and the inconsistent reporting of the germination process, sometimes only measured by the number of germinated seeds, hinder the inclusion of a method into official rules (Santana et al., 2012), added to the difficulty of meeting principles of metrology (Kataoka et al., 2011).

It is noteworthy that in the Rules for Seed Testing, in its latest issue (Brasil, 2009), there are methods proposed for germination tests in 1365 species. Of these, just over 276 are forest and shrub species (Ferraz and Calvi, 2011), mostly not native to Brazil. In order for a method for germination test to be validated and included in the official rules, procedures proposed by ISTA (2007) must be followed. It is a collaborative process between the laboratories, to ensure that the procedure provides reliable and reproducible results. To be validated, the method has to have accuracy, robustness, reproducibility and repeatability (Kataoka et al., 2011). The manual of ISTA brings statistical techniques that are widely used and robust, but that do not restrict innovation. As the process involves a factorial design, consisting of laboratories and plots, one of the measures of statistical precision that can be used in the evaluation of the process is the coefficient of variation (*CV*). This measure allows the comparison of accuracy among experiments without the need to have equal units (Amaral et al., 1997), for characterizing the relative dispersion of data in relation to the mean value (Santana and Ranal, 2000).

To study the coefficient of variation of the same character in different experiments, the researcher must have experience with the variable (Steel and Torrie, 1980), although bands for classification have been proposed (Pimentel-Gomes, 2000). However, this classification is too broad and does not consider the peculiarities of the species studied, and mainly makes no distinction as to the nature of the trait (Garcia, 1989; Costa et al., 2002).

Several articles have demonstrated the need to establish the limits for classification of *CV* for agricultural crops of interest (Amaral et al., 1997; Costa et al., 2002; Carvalho et al., 2003), for forage species (Ambrosano and Schammas , 1994; Clemente and Muniz, 2002) and for the silvicultural characters of forest species of the genus *Pinus* and *Eucalyptus* (Garcia, 1989). For forest seeds, there is no rating scale; the measure is only used to ascertain the reliability of the experiment. The aim was to compare and discuss, through the coefficient of variation for normal seedlings, the variability present in the process of validation of methods obtained in the germination test of seeds of 20 species belonging to the family Fabaceae.

Material and Methods

The study used data from the process that resulted in the validation of methods for testing the germination of seeds of 20 tree species of the family Fabaceae. The family Fabaceae was chosen on the basis of available literature on dormancy and germination of seeds, and the availability for purchase, donation and seed collection. The details of the method with information on pre-germination treatments, scores, substrate, temperature and photoperiod are in Table 1. Before the formation of lots, number of samples in seven to 16 (Table 1) were formed from seeds of at least five sources, with at least four matrices. The plots, at least three, were formed by mixing the samples with seed quality physiological to generate similar variability, as well as similarity in size and shape. In lots of randomization sent to each lab (minimum six per species) were provided for four eight replications of 50 seeds, totaling 200 and 400 seeds, respectively. Moreover, even the laboratories have evaluated several features of germination of normal seedlings was examined only for purposes of validation.

In a preliminary analysis the test of Box-plot was applied, eliminating the outliers, known as "outliers" according to the Manual of Validation (ISTA, 2007). To test the assumptions of the variance analysis model, the Shapiro-Wilk test was used for the normality of model residuals, and Levene for the homogeneity of variances, both at 0.01 significance level. When both assumptions were met, the F test of Snedecor was applied, also at 0.01 of significance for the main effects (plot and lab) and for interaction. When at least one of the assumptions was not met, the percentage of normal seedlings were transformed by arcsine$\sqrt{x/100}$ and assumptions were tested again. From the model of analysis, the experimental coefficient of variation was determined by the equation: $CV_{experimental} = (\sqrt{QMR}/\bar{y}...)100$; where *QMR:* is the mean square of the residual, and $\bar{y}...$: is the general mean of the experiment. The coefficient of variation per plot was found using the equation: $CV_{lot} = (s_{i.}/\bar{y}_{i.})100$, where $S_{i.}$: is the standard deviation of normal seedling percentage obtained from the *i*-th plot, and $\bar{y}_{i.}$: is the normal seedling

percentage mean obtained from the *i*-th plot. The coefficient of variation per lab was determined using the equation $CV_{laboratory} = (s_{j.}/\bar{y}_{j.})100$, where $S_{j.}$: is the standard deviation of normal seedling percentage obtained from the *j*-th lab, and $\bar{y}_{.j.}$: is the normal seedling percentage mean obtained from the *j*-th lab.

Table 1. Methods for testing the germination of the seeds of 20 species of the family Fabaceae used in the validation process, including common name, subfamily and national record number of cultivars (RNC).

Species/Common name Famíly/RNC	samples	Summary of method		Additional instructions including recommendations to overcome dormancy	Bibliography
Acacia polyphylla D.C./ Acácia-monjolo Fabaceae-Mimosoideae/ RNC: 23371	11	Substrate/ arrangement	Filter/ roll paper	Detergent solution wash[1]	Silva et al. (2007)
		Temperature/ Light	25 ºC/ Continuous		
		Count (days)	7; 14		
Albizia hassleri (Chodat) Burkart/Albízia-farinha-seca Fabaceae-Mimosoideae/ RNC: 23390	8	Substrate/ arrangement	Filter/ roll paper	Fumigation with 0.025% NaClO [2] for 2 ', before and after clipping on the upper side without reaching the cotyledons	Gonzales et al. (2010)
		Temperature/ Light	25 ºC/Continuous		
		Count (days)	7; 14		
Anadenanthera macrocarpa (Benth.) Brenan/ Angico-monjolo Fabaceae-Mimosoideae/ RNC: 23423	10	Substrate/ arrangement	Filter/ roll paper	Detergent solution wash[1] Sowing in drier paper	Souza and Lima (1985)
		Temperature/ Light	25 ºC/Continuous		
		Count (days)	4; 10		
Apuleia leiocarpa (Vogel) J. F. Macbr./ Garapeira Fabaceae-Caesalpinioideae/ RNC: 23475	7	Substrate/ arrangement	Filter/ roll paper	Fumigation with 0.25% NaClO [2] for 2 ', clipped on the upper side without reaching the cotyledons, followed by fumigation with 0.025% NaClO[2]	Henicka et al. (2006)
		Temperature/ Light	25 ºC/Continuous		
		Count (days)	7; 10		
Dalbergia miscolobium Benth./Caviúna-do-cerrado Fabaceae-Papilionoideae/ RNC: 23689	8	Substrate/ arrangement	Filter/ roll paper	Detergent solution wash[1] followed by fumigation with 0.025% NaClO[2] per 2'	Braz et al. (2000)
		Temperature/ Light	25 ºC/Continuous		
		Count (days)	7; 10		
Enterolobium contortisiliquum (Vell.) Morong/ Tamboril-da-mata Fabaceae – Mimosoideae/ RNC: 24025	15	Substrate/ arrangement	Filter/ roll paper	Scarification in the region opposite to the micropyle with iron sandpaper [3] No. 50, without reaching the cotyledons, and then washed with detergent solution [1]	Silva and Santos (2009)
		Temperature/ Light	25 ºC/Continuous		
		Count (days)	7; 14		
Hymenaea courbaril L./ Jatobá Fabaceae-Caesalpinioideae/ RNC: 24177	12	Substrate/ arrangement	Filter/ roll paper	Fumigation with 0.025% NaClO [2] for 2 ', before and after scarification at the opposite end to the micropyle with iron sandpaper [3] No. 100 and soaked for 24 hours; moisten substrate in the seventh day after sowing	Almeida et al. (1999)
		Temperature/ Light	25 ºC/Continuous		
		Count (days)	21; 28		
Mimosa caesalpiniaefolia Benth./ Sansão-do-campo Fabaceae-Mimosoideae /RNC: 12505	13	Substrate/ arrangement	Filter/ roll paper	Washing with detergent solution before and after clipping on the upper side without reaching the cotyledons	Novembre et al. (2007)
		Temperature/ Light	25 ºC/Continuous		
		Count (days)	5; 10		
Mimosa scabrella Benth./ Bracatinga-comum Fabaceae-Mimosoideae/ RNC: 23423	9	Substrate/ arrangement	Filter/ roll paper	Fumigation with 0.05% NaClO [2] for 2 ', followed by wet heat treatment at 80 ° C and remaining at pre-soak for 24 hours at room temperature	Barazetti and Sccoti (2010)
		Temperature/ Light	25 ºC/ Continuous		
		Count (days)	5; 10		
Ormosia arborea (Vell.) Harms/ Tento-vermelho Fabaceae-Papilionoideae/ RNC: 24547	14	Substrate/ arrangement	Filter/ roll paper	Fumigation with 0.05% NaClO for 2 ', before and after side scarification on the upper red portion with water sandpaper [3] No. 150, without reaching the cotyledons, followed by pre-soaking for 24 hours	Marques et al. (2004)
		Temperature/ Light	25 ºC/Continuous		
		Count (days)	21; 28		
Parapiptadenia rigida (Benth.) Brenan/ Angico-vermelho Fabaceae-Mimosoideae/ RNC: 24547	9	Substrate/ arrangement	Filter/ roll paper	Detergent solution wash[1]	Vaz Mondo et al. (2008)
		Temperature/ Light	25ºC/Continuous		
		Count (days)	7; 14		

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Parkia pendula (Willd.) Benth. ex Walp/ Visgueiro-bolota Fabaceae-Mimosoideae/ RNC: 24554	11	Substrate/ arrangement Temperature/ Light Count (days)	Filter/ roll paper 30 ºC/ Continuous 7; 14	Fumigation with 0.05% NaClO ² for 2 ', before and after clipping on the upper side of seed without reaching the cotyledons	Rosseto et al. (2009)
Peltogyne confertiflora (Mart. ex Hayne) Benth./ Pau-roxo-da-várzea Fabaceae- Caesalpinioideae/ RNC: 24565	9	Substrate/ arrangement Temperature/ Light Count (days)	Filter/ roll paper 25 ºC/Continuous 21; 28	Fumigation with 0.05% NaClO² for 2' and pre-soaking for 24 hours	Silva et al. (2009)
Peltophorum dubium (Spreng.) Taub./Canafistula-branca Fabaceae- Caesalpinioideae/ RNC: 23304	11	Substrate/ arrangement Temperature/ Light Count (days)	Filter/ roll paper 25 ºC/Continuous 7; 14	Clipping in the area opposite to the micropyle, without reaching the cotyledons, and washing with detergent solution ¹	Oliveira et al. (2008)
Plathymenia reticulata Benth./Vinhático-do-campo/ Fabaceae-Mimosoideae RNC: 24607		Substrate/ arrangement Temperature/ Light Count (days)	Filter/ roll paper 25 ºC/Continuous 10; 16	Fumigation with 0.5% NaClO ² for 2', and then side clipping on the upper portion, without reaching the cotyledons, and subsequently fumigated with 0.025% NaClO ² for 2 '	Lacerda et al. (2004)
Pterogyne nitens Tul./ Pau-amendoim Fabaceae- Caesalpinioideae/ RNC: 25362	10	Substrate/ arrangement Temperature/ Light Count (days)	Filter/ roll paper 25 ºC/Continuous 7; 14	Fumigation with 0.025% NaClO ² for 2 ', and then side clipped on the upper portion and subsequently washed with detergent solution ¹	Nassif and Perez (2000)
Schizolobium parahyba var. *amazonicum* (Huber ex Ducke) Barneby/ Paricá Fabaceae-Caesalpinioideae/ RNC: 25496	8	Substrate/ arrangement Temperature/ Light Count (days)	Filter/ roll paper 25 ºC/Continuous 7; 10	Fumigation with 0.025% NaClO² for 2 ', before and after scarification on the opposite end to the micropyle with iron sandpaper ³ No. 80, followed by pre-soaking for 24 hours. Before sowing, the seeds must be rubbed on sieve to remove the waxy cuticle and then washed with detergent solution ¹	Ramos et al. (2006)
Senna macranthera (Dc. ex Collad.) H. S. Irwin & Barneby/ Sena-fedegosão Fabaceae-Caesalpinioideae/ RNC: 25516	16	Substrate/ arrangement Temperature/ Light Count (days)	Filter/ roll paper 25 ºC/ Continuous 7; 14	Fumigation with 0.05% NaClO ² for 2 ', before and after clipping on the upper side without reaching the cotyledons	Lemos Filho et al. (1997)
Senna multijuga (Rich.) H. S. Irwin & Barneby/ Sena-multijuga Fabaceae- Caesalpinioideae/ RNC: 25517	12	Substrate/ arrangement Temperature/ Light Count (days)	Filter/ roll paper 25 ºC/Continuous 4; 7	Fumigation with 0.025% NaClO² for 2 ', before and after clipping the area opposite to the micropyle using a nail clipper	Lemos Filho et al. (1997); Lacerda et al. (2004)
Stryphnodendron polyphyllum Mart./Barbatimão-polifilo Fabaceae-Mimosoideae/ RNC: 24640	9	Substrate/ arrangement Temperature/ Light Count (days)	Filter/ roll paper 25 ºC/Continuous 10; 14	Fumigation with 0.025% NaClO² for 2 ', before and after side clipping in the middle portion using a nail clipper	Lemos Filho et al. (1997)

¹Detergent: ratio of five drops per 100 mL of water, from 5 to 10 minutes, followed by tap wash and then left in distilled water for 3 minutes; ² NaClO: after sterilization with sodium hypochlorite seeds were washed in tap water to remove excess solution and then immersed in distilled water for 3 minutes; ³ The sandpaper number indicates the mean mineral grain size with which the sandpaper has been manufactured, where the higher the number, the smaller the grain size, and the thinner the sandpaper (composition of sandpaper: paper plating, aluminum oxide and adhesive, iron sandpaper composition: tarp plating, aluminum oxide and resin).

Results and Discussion

The synthesis of the statistical analysis of the seed germination test method validation of 20 native species of the family Fabaceae showed no significant laboratory effect, as well as interaction, and significant plots for the percentage of normal seedlings for all species (Table 2). The biggest impact of this result was the finding that even when seeds were subjected to specific pre-germinal treatments, such as clipping (lopping), and scarification, among others, the results were similar in different laboratories. According to the Manual of Validation of the International Seed Testing Association (ISTA, 2007), the goal of validation is to obtain comparable results among laboratories. The *F* statistics for the main effects and interaction were applied without neglecting the residual normality assumptions of the model and the homogeneity of variances. The statistical analysis

was entirely based on the Manual of Validation of the International Seed Testing Association (ISTA, 2007).

Table 2. Summary of the analysis of variance for normal seedling percentages of the seed germination test method validation process of 20 Fabaceae species.

Species	Seeds	Mean Square[1]		
		Lab	Lot	Interaction
Acacia polyphylla	non dormant	53.208^{ns}	5851.018**	15.087^{ns}
Albizia hasslerii	dormant	44.813^{ns}	9443.708**	21.424^{ns}
Anadenanthera macrocarpa	non dormant	135.749^{ns}	16278.010**	78.963^{ns}
Apuleia leiocarpa	dormant	223.240^{ns}	4243.907**	47.755^{ns}
Dalbergia miscolobium	non dormant	289.570^{ns}	6347.566**	69.283^{ns}
Enterolobium contortisiliquum	dormant	32.672^{ns}	32558.149**	30.107^{ns}
Hymenaea courbaril	dormant	127.116^{ns}	9650.608**	49.827^{ns}
Mimosa caesalpiniaefolia	dormant	19.049^{ns}	10635.189**	11.055^{ns}
Mimosa scabrella	dormant	94.321^{ns}	5265.448**	62.586^{ns}
Ormosia arborea	dormant	173.933^{ns}	7330.167**	198.700^{ns}
Parapiptadenia rigida	non dormant	40.622^{ns}	13933.930**	46.897^{ns}
Parkia pendula	dormant	197.792^{ns}	29079.500**	105.917^{ns}
Peltogyne confertiflora	non dormant	14.489^{ns}	10940.129**	10.065^{ns}
Peltophorum dubium	dormant	84.201^{ns}	14515.685**	73.511^{ns}
Platymenia reticulata	dormant	197.014^{ns}	17253.18**	43.197^{ns}
Pterogyne nitens	dormant	87.413^{ns}	7532.157**	63.359^{ns}
S. parahyba var. amazonicum	dormant	44.396^{ns}	6641.005**	77.340^{ns}
Senna macranthera	dormant	107.763^{ns}	11111.090**	32.593^{ns}
Senna multijuga	dormant	85.953^{ns}	11696.995**	15.357^{ns}
Stryphnodendron polyphyllum	dormant	82.964^{ns}	14176.043**	15.895^{ns}

$^{**; ns}$ significant and non-significant, respectively at 0.01 of significance by ANOVA *F-test*.

The experimental coefficients of variation (*CV*) (model with main effects and interaction) for normal seedlings were low (up to 9.84%) to medium (up to 17.66%), with no records of high *CV* (20 <*CV* ≤ 30%) nor very high (*CV*> 30%) (Table 3). The low and medium values of *CV sensu* Pimentel-Gomes (2000) showed accuracy in the variation of repetitions of same laboratory and plot combination, thus, it can be inferred that the proposed methods for testing the germination of the seeds of these species had repetitiveness. Residual normality assumptions and homoscedasticity were treated to the percentage of normal seedlings for all species, some with data transformation of the angular type. Species with low coefficients of variation for normal seedlings, such as *Acacia polyphylla*, *Enterolobium contortisiliquum* and *Mimosa caesalpiniaefolia*, showed heterogeneous variances and therefore required transformation (Table 4). However, species with the highest coefficients of variation were *Mimosa scabrella*, *Dalbergia miscolobium* and *Ormosia arborea* with 15.32 and 17.40 and 17.66%, respectively; variances were homogeneous with the original data and did not require data transformation. This revealed that the *CV* was no evidence capable of predicting the heterogeneity of variances and, consequently, there is a need to transform data.

Data transformations reduce the scale of values and thus are more appropriate where the problem is heterogeneity of variances and not normality, since the latter reflects the behavior of the species (Santana and Ranal, 2000). Thus, meeting the assumption of normality is a desirable goal, but not the main one, because of the robustness of the linear models to non-normality, however sensitive to heteroscedasticity (Faraway, 2006). In the literature, several transformations have been suggested for data expressed as proportions or percentages (Piepho, 2003) and aim to break the dependency between mean and variance, which is characteristic of this type of variable (Santana and Ranal, 2000). When used arbitrarily, it results in undesirable effects, since there is no guarantee of the non-violation of the assumptions of the analysis of variance model (Sileshi, 2012). In addition, a transformation that corrects the violation of an assumption could result in the violation of another (Sileshi, 2007).

Of the 20 native species, only *Peltogyne confertiflora* presented residuals with non-normal distribution for the percentages of normal seedlings. Once transformed, the characteristic met the assumption (Table 3). However, some authors have reported that non-normality is prevalent in ecological data (Warton and Hui, 2011) and will likely be the rule and not the exception in germination (Sileshi, 2012). Unlike most major crops, native forest species have great genetic

diversity (Sarmento and Villela, 2010). Forest trees mostly reproduce by crosses; they occupy large geographical areas and different "habitats", they are then expected to have high levels of genetic variation (Hamrick and Loveless, 1986). In fact, studies on the reproductive systems of tropical tree species, by isozymes, have shown that the vast majority is alogamous or have mixed system, predominantly alogamous (Murawski, 1995). Their wide geographical distribution, type of reproduction and interactions between genetic and environmental factors lead to great diversity, also noted in the physiological characteristics of seeds and in their germination process (Rego et al., 2005).

Table 3. Coefficients of variation for the percentages of normal seedlings of the germination test methodology validation process of 20 species of the family Fabaceae, including the assumptions of the model and precision of the experiment, with adjectives.

Species	*CV* (%)		Statistics[2]	
	Original (adjective[1])	arcsine $\sqrt{x/100}$ (adjective[1])	*W*	*F*
Acacia polyphylla	9.84 (low)	7.58 (low)	0.986	2.168
Albizia hasslerii	11.41 (medium)	-	0.944	0.944
Anadenanthera macrocarpa	13.30 (medium)	-	0.993	1.298
Apuleia leiocarpa	11.67 (medium)	-	0.969	1.965
Dalbergia miscolobium	17.40 (medium)	-	0.983	1.010
Enterolobium contortisiliquum	8.82 (low)	9.56 (low)	0.988	1.829
Hymenaea courbaril	9.55 (low)	-	0.983	0.614
Mimosa caesalpiniaefolia	6.49 (low)	7.55 (low)	0.991	2.092
Mimosa scabrella	15.32 (medium)	-	0.975	1.278
Ormosia arborea	17.66 (medium)	-	0.982	1.306
Parapiptadenia rigida	12.84 (medium)	-	0.971	2.161
Parkia pendula	14.85 (medium)	-	0.989	1.074
Peltogyne confertiflora	8.83 (low)	6.52 (low)	0.978	1.230
Peltophorum dubium	10.46 (medium)	-	0.977	1.039
Platymenia reticulata	13.05 (medium)	-	0.982	1.871
Pterogyne nitens	10.69 (medium)	-	0.975	0.566
S. parahyba var. amazonicum	7.60 (low)	-	0.986	1.788
Senna macranthera	14.03 (medium)	-	0.988	1.287
Senna multijuga	9.54 (low)	-	0.985	1.661
Stryphnodendron polyphyllum	11.87 (medium)	-	0.975	2.026

[1]adjectives *sensu* Pimentel-Gomes (2000); [2]*W* and *F*: statistics from the Shapiro-Wilk and Levene tests, values in bold indicate normality of residuals and homogeneity of variances, respectively.

Normal distribution essentially shapes random events, which individual occurrence does not follow rules or standards. Because they do not have genetic improvement and are susceptible to several environmental factors, randomness was present in the germination test method validation process of 20 species, justifying the normal distribution of residuals. In summary, research has proved theory. Santana et al. (2012), when working on validation of methods for testing the germination of seeds of 10 species native to Brazil, have found that for all species the model residuals for normal seedlings had normal distribution and variances were homogeneous. The *F* statistic showed no differences in the percentage of normal seedlings among laboratories, nor interaction between laboratory and plot, only differences in the percentages of plots were noted (Table 2). In fact, plots were formed to provide different percentages of normal seedlings, which was confirmed by analysis of variance. It is consistent that seeds from plots with different qualities tend to have different responses to the method (Oliveira et al., 2005; Kataoka et al., 2011). Martins and Nakagawa (2008) found that plots may respond differently to pre-germination treatments. However, the methods have been extensively tested and validated after proved to be robust, ie efficiency ought to be maintained regardless of plot quality. The ideal method should withstand adverse conditions of application and be also precise and accurate for detecting subtle differences in quality (Waeny, 1980). Accordingly, one should prioritize treatments for breaking efficient cutaneous dormancy, which are independent of plot origin (Smiderle and Souza, 2003; Borges et al., 2004).

Except for *Acacia polyphylla*, *Anadenanthera macrocarpa*, *Dalbergia miscolobium*, *Parapiptdenia rigida* and *Peltogyne confertiflora,* which have not had dormant seeds, the seeds of other species underwent treatments to overcome dormancy. The methods of seed dormancy overcoming did not cause great variations among repetitions, considering that coefficients of variation were medium sensu (Pimentel-

Gomes, 2000), reaching a maximum of 17.66% (Table 3). No difficulty of standardizing expected, due to differences in the intensity of scarification and lopping, control of heat treatment temperature, among other factors, was found.

The coefficients of variation per plot were below 10% for high quality plots of twelve species, and over 20% for low-quality plots of nine species (Table 4). This result indicated that as plots quality decreased, the classification of normal seedlings became doubtful and coefficients of variation increased. Kataoka et al. (2011) noted that plot coefficient of variation for seeds in advanced stages of deterioration, ie, lower quality, showed the highest values. Although, normal seedlings of *Mimosa caesalpiniaefolia* and *Peltogyne confertiflora*, with dormant and non-dormant seeds, respectively, had plot coefficients of variation below 10% regardless of quality.

Table 4. Coefficient of variation for normal seedlings per plot formed for the germination test methodology validation process of 20 species of the family Fabaceae.

Species	Quality of plot					
	High		intermediate		low	
	CV (%)	Seedlings normal (%)	*CV* (%)	Seedlings normal (%)	*CV* (%)	Seedlings normal (%)
Acacia polyphylla[1]	6.94	89.50	7.94	62.71	10.18	42.63
Albizia hasslerii	10.41	64.25	7.96	54.39	17.51	30.72
Anadenanthera macrocarpa	10.01	76.22	17.67	46.75	22.08	31.75
Apuleia leiocarpa	7.64	86.45	12.43	72.00	16.99	59.63
Dalbergia miscolobium	12.31	72.63	23.74	50.23	22.57	41.00
Enterolobium contortisiliquum[1]	7.16	95.27	9.30	46.09	20.38	16.43
Hymenaea courbaril	7.44	84.27	11.49	70.00	15.57	41.90
Mimosa caesalpiniaefolia[1]	6.20	91.25	9.35	58.13	5.92	34.89
Mimosa scabrella	13.68	60.55	18.70	46.83	14.43	37.26
Ormosia arborea	16.84	62.75	18.73	47.83	33.85	27.92
Parapiptadenia rigida	16.50	95.04	10.18	58.63	8.59	49.50
Parkia pendula	8.45	88.88	20.90	50.63	41.56	19.28
Peltogyne confertiflora[1]	3.65	96.00	8.05	60.79	8.97	37.61
Peltophorum dubium	8.35	75.06	12.23	54.13	13.44	31.63
Platynenia reticulata	7.05	88.96	18.82	56.58	20.74	35.75
Pterogyne nitens	10.81	71.17	34.97	51.74	36.66	35.79
S. parahyba var. *amazonicum*	1.66	97.72	9.62	76.86	12.70	65.68
Senna macranthera	12.01	69.78	10.13	53.96	23.75	32.23
Senna multijuga	4.05	89.65	11.77	56.75	15.59	42.79
Stryphnodendron polyphyllum	6.89	81.48	12.63	62.17	20.60	32.50

[1]Data transformed by arcsine $\sqrt{x/100}$, where x is the percentage of normal seedlings, *CV*: coefficient of variation.

Still on the coefficient of variation per plot (Table 4), no significant relationship was found between the coefficient of variation and treatments for breaking dormancy, because even species with non-dormant seeds, such as *Anadenanthera macrocarpa* and *Dalbergia miscolobium,* showed high coefficients of variation (from 20 to 30%) for low quality plots. Similarly, *Schizolobium parahyba* var. *amazonicum*, a species which seeds have high degree of tegumentar dormancy, had the lowest coefficient of variation per plot, 1.66% for normal seedlings from the plot of higher quality.

The coefficients of variation estimated by laboratory (Table 5) were higher than by plot (Table 4), for covering the spectrum of germination conferred by the three plots with different qualities. However, this variation was uniform for all laboratories, indicating that the proposed method for testing seed germination of species of the family Fabaceae was reproducible. The coefficient of variation is a comparative measure of the existing variability between two experiments regarding factors that are not controlled, but never on the quality of the assay (Ranal and Santana, 2000). The differences in the magnitudes of lab coefficient of variation among species occurred primarily due to differences among plots. Lab coefficients of variation for normal seedlings of *Parkia pendula* were the highest among the species, but such results cannot be associated with the species. Variations in seed quality, from 19.3% (low quality) to 88.9% (high quality), have overestimated the coefficient of variation. In contrast, *Schizolobium parahyba* var. *amazonicum* low lab coefficients of variation were due in part to smaller difference in the quality of plots (from 97.7 to 65.7%).

Table 5. Lab coefficient of variation of the germination test methodology validation process of 20 species of the family Fabaceae.

	Species									
Lab	*Acacia polyphylla*	*Albizia hasslerii*	*Anadenanthera macrocarpa*	*Apuleia leiocarpa*	*Dalbergia miscolobium*	*Enterolobium contortisiliquum*	*Hymenaea courbaril*	*Mimosa caesalpiniaefolia*[1]	*Mimosa scabrella*	*Ormosia arborea*
1	-	-	36.89	-	29.99	48.55	28.63	30.51	25.80	-
2	-	40.31	28.56	-	31.78	56.13	33.51	28.47	22.75	-
3	24.00	37.55	-	-	32.16	-	-	30.73	-	-
4	28.30	-	-	-	-	46.68	25.13	28.61	28.01	32.10
5	-	-	37.58	-	-	47.93	28.15	32.29	21.00	42.52
6	26.00	46.19	31.17	20.91	29.12	43.75	-	-	-	31.25
7	23.40	41.69	28.38	24.91	-	46.78	-	31.86	35.36	46.75
8	-	-	-	-	-	53.71	-	29.45	20.29	-
9	-	37.73	34.55	-	26.34	54.16	-	-	23.71	-
10	-	30.16	24.68	16.92	-	48.77	-	-	22.52	-
11	23.12	41.14	35.38	17.61	24.43	-	-	-	23.57	24.27
12	-	-	-	16.84	-	50.90	-	-	-	-
13	-	-	-	18.05	-	56.38	-	32.12	-	-
14	26.39	42.49	-	-	-	-	25.13	-	32.77	-
15	-	-	-	-	-	-	-	-	-	45.69
16	-	-	-	-	-	-	31.91	-	-	-

	Species									
Lab	*Parapiptadenia rigida*	*Parkia pendula*	*Peltogyne confertiflora*[1]	*Peltophorum dubium*	*Platymenia reticulata*	*Pterogyne nitens*	*Schizolobium parahyba var. amazonicum*	*Senna macranthera*	*Senna multijuga*	*Stryphnodendron polyphyllum*
1	-	-	32.75	34.52	-	-	15.09	39.36	33.36	36.55
2	46.53	-	32.24	32.58	45.28	34.64	-	36.02	33.76	-
3	-	-	32.95	-	38.77	-	-	34.62	-	36.07
4	-	61.23	-	-	42.48	35.50	19.94	29.98	-	-
5	46.41	61.98	-	-	-	-	23.67	-	-	-
6	-	-	32.84	37.02	-	-	17.63	-	29.45	-
7	-	-	33.76	-	-	31.38	20.22	30.86	33.58	38.40
8	-	-	-	-	-	-	-	-	-	-
9	-	59.31	-	-	36.30	28.72	16.90	32.61	37.62	-
10	47.69	65.49	-	31.01	36.99	25.20	-	-	32.25	39.23
11	48.38	-	-	37.32	39.28	-	17.39	36.20	-	33.29
12	-	-	-	32.30	-	-	-	33.49	-	-
13	48.15	-	-	-	-	28.40	-	-	-	-
14	45.02	55.46	-	-	-	-	-	-	-	41.67
15	-	46.08	33.50	44.04	-	-	-	-	-	-
16	-	-	30.03	-	-	-	-	-	-	-

[1]Data transformed by arcsine $\sqrt{x / 100}$, where x is the percentage of normal seedlings; *CV*: Coefficient of variation.

Conclusions

For normal seedlings of 20 Brazilian forest species, the coefficients of variation vary from low (up to 9.84%) to medium (up to 17.66%), contrary to what is expected from the great genetic diversity of these species.

The increased coefficient of variation is not related to the treatment of seed dormancy overcoming, however, it increases as plot quality decreases.

The high coefficients of variation estimated by laboratory,

and overestimated by the effect of plots, are uniform, which indicates that the methods are reproducible.

The coefficient of variation is no evidence capable of predicting the heterogeneity of variances and, consequently, there is a need to transform data.

Randomness is present in the process of method validation of the 20 forest species of the family Fabaceae, due to the normal distribution of residuals.

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