Electrical conductivity test in *Melanoxylon brauna* Schott. seeds (Fabaceae-Caesalpinioideae)

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ABSTRACT

The electrical conductivity test is a simple and rapid method for evaluating seed vigor. The aim of the present study was to optimize the electrical conductivity test for assessing the vigor of *Melanoxylon brauna* seeds by investigating the effects of seed number (25, 50, or 75), water volume (25, 50, or 75 mL), and soaking duration (24, 48, or 72 h). The seeds belonged to two lots (I and II) that were collected in 2010 and 2012, respectively, and were incubated in a germination chamber at 25 ºC. Electrical conductivity was determined using a conductivity meter Micronal model B220. The Lot II seeds performed better, in terms of vigor and germination, than the Lot I seeds, regardless of other parameters. In addition, electrical conductivity decreased with increasing water volume and soaking duration, regardless of seed lot. In order to most effectively evaluate the physiological quality of *M. brauna* seed lots, the electrical conductivity test should be performed using 50 seeds, 50 mL of water, and a 48-h soaking period.

Keywords: Vigor. Germination. Forestry seeds. Brauna.

Teste de condutividade em sementes de *Melanoxylon brauna* Schott. (Fabaceae-Caesalpinioideae)

RESUMO

O teste de condutividade elétrica tem sido utilizado para avaliar o vigor das sementes e apresenta como vantagens fácil condução e possibilidade de inferência sobre o uso e manejo das sementes em curto espaço de tempo. Este trabalho objetivou avaliar diferentes metodologias para o teste de condutividade elétrica, com variações na quantidade de sementes utilizadas, volume de água e tempo de embebição para determinação do vigor de sementes de *Melanoxylon brauna*. Foram utilizadas sementes pertencentes a dois lotes, coletadas nos anos 2010 e 2012, os quais foram denominados lote I e lote II, respectivamente. Para o teste de condutividade elétrica (CE) foram testadas as combinações de 25, 50 e 75 sementes, em volumes de 25, 50 e 75 mL de água destilada, nos tempos 24, 48 e 72 horas de embebição, sendo as sementes pesadas e incubadas em câmara de germinação em temperatura constante de 25 ºC. A CE foi determinada com condutivímetro Micronal modelo B220. Observou-se melhor desempenho das sementes do lote II em relação ao lote I, tanto nos testes de germinação quanto nos de vigor, em todas as combinações testadas. Houve tendência de decréscimo nos valores de CE na medida em que se aumentou a quantidade de água e o tempo de embebição das sementes, para os dois lotes avaliados. Para diferenciar lotes de sementes de *Melanoxylon brauna* quanto à qualidade fisiológica, o teste de condutividade elétrica deve ser conduzido no tempo de 48 horas de embebição, com 50 sementes e 50 mL de água.

Palavras-chave: Vigor. Germinação. Sementes florestais. Braúna.

1. Introduction

The restoration of damaged forestry ecosystems and maintenance of biodiversity has recently become a major focus of government policies and social interest, and in the Atlantic Forest, *Melanoxylon brauna*, a high-value arborous species that is used in construction and landscaping, has received particular interest (Lorenzi, 2009). Currently, *M. brauna* is categorized as "vulnerable" on the "list of Brazilian flora species threatened of extinction" (MMA, 2008).

The propagation of *M. brauna* is by seeds, and the analysis of seed quality is important for seedling production. Physiological quality, which can be measured as germination capacity or seed vigor, is essential for the use and preservation of seed lots. According to Rajjou et al. (2012), seed vigor can be defined as the intrinsic characteristics of specific seed lots that determine the capacity of seed lots in the field, under a large set of environmental conditions.

Electrical conductivity is a measure of exudates leached by seeds during a soaking period and is based on the principle that cell membranes are restored slower in damaged seeds and that more solutes (e.g., sugars, amino acids, fatty acids, proteins, enzymes, and organic ions) are lost into aqueous solutions by damaged seeds than by vigorous seeds (Marcos Filho, 2005).

However, the methodology of electrical conductivity tests often differs, depending on the plant species being examined. The assessment of seed vigor can be affected by seed lot and seed number, as well as by soaking duration and temperature (Vieira and Kryzanowski, 1999. Therefore, it is important to investigate optimal methods, when information is lacking, especially for forestry important species.

According to Vieira and Kryzanowski (1999), the electrical conductivity test is a simple and practical method for assessing seed vigor and for rapidly providing insight into the proper use and management of seed lots.

The aim of the present study was to optimize the electrical conductivity test for assessing the vigor of *M. brauna* seeds by investigating the effects of seed number, water volume, and soaking duration.

2. Material and Methods

The present study was conducted in the Forestry Seeds Analysis Laboratory of the Federal University of Viçosa, Brazil, from October 2012 to January 2013, using two groups of *M. brauna* seeds (lots I and II) from fruits that were collected from trees in Leopoldina, MG, Brazil, in September 2010 and 2012, respectively. After collection, the fruits were dried in the sun, and the seeds were manually removed. During this procedure, immature, rotten, and damaged seeds were removed, and the selected seeds were stored in fiber drums in a cold chamber (5 °C and 60% relative moisture) until the initiation of the experiments.

Seeds from both lots were germinated over 10 days in Petri dishes with germitest paper, in a germination chamber at 25ºC and constant light. Germination was calculated daily as the number of seeds with newly emerged radicals, and the germination speed index (GSI) was calculated using the formula proposed by Maguire (1962).

Electrical conductivity tests were performed using different combinations of seed number (25, 50, or 75), water volume (25, 50, or 75 mL), and soaking duration (24, 48, or 72 h). For each combination, the seeds were weighed using a 0.01 g precision balance, transferred to disposable cups that contained the appropriate volume of distilled water, and incubated in a germination chamber that was maintained at 25 ºC.

After soaking, the electrical conductivity of each replicate was measured using a B 330 MICRONAL conductivity meter, with K = 1.0 and results were expressed in μS cm^{-1} g^{-1}. Each treatment was represented by five replicates.

The experimental design was randomized, with a 3×3×3 factorial scheme (seed quantity, distilled water volume, and soaking duration). After verifying data normality, using the Kolmogorov-Smirnov and Lilliefors tests, and variance homogeneity, using the Cochran and Bartley tests, the data were subject to analysis of variance (ANOVA). The averages of germination, GSI, and electrical conductivity, between both lots was provided by the Tukey test at 5% of probability.

Correlation between electrical conductivity values and germination and GSI were investigated using the F-test, at 5%, in Statistica 8.0 (Statsoft, 2009).

3. Results and Discussion

The average percent germination of the Lot I seeds (54%) was significantly less than that of the Lot II seeds (92%; Figure 1a), and a similar pattern was observed for GSI, with the Lot II seeds being more vigorous (Figure 1b). These differences indicated that the Lot I seeds, collected in 2010, were less viability and vigorous that the Lot II seeds, collected in 2012.

According to Bewley et al. (2013), seed deterioration is affected by environmental conditions (temperature and relative moisture), genetics (species, seed lot, or initial seed quality), and the presence of fungi or bacteria, and the physiological and biochemical alterations that occur in seeds during deterioration are associated with tissue damage and reduced germination capacity.

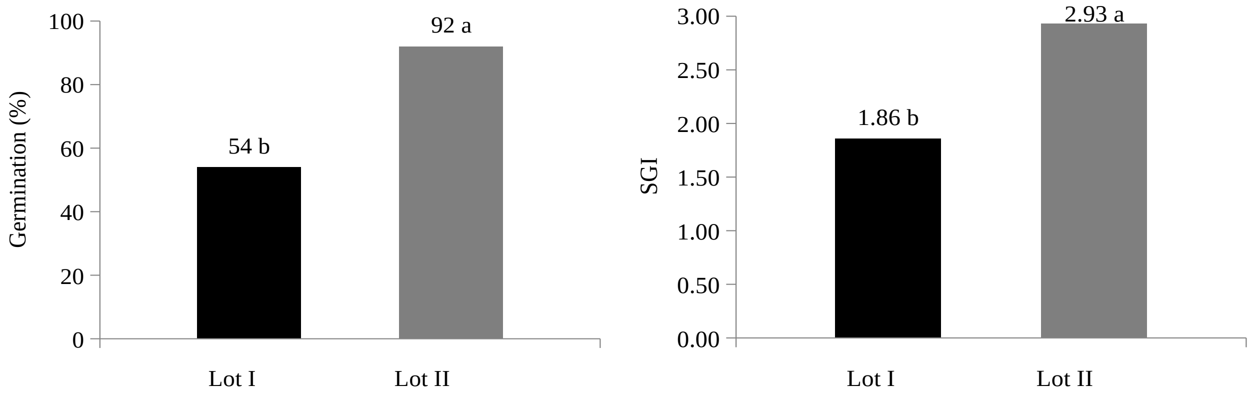

Figure 1 - Germination (a) and germination speed index (GSI) (b) of *Melanoxylon brauna* seeds of lots I and II, collected in the years 2010 and 2012, respectively.

The disruption of membrane system was observed in *Phaseolus vulgaris* seeds (Lee et al., 2012), and the increase of lipid peroxidation and decreasing of enzyme activities from the antioxidant complex was observed in *M. brauna* (Corte et al., 2010), resulting on seed quality loss.

The electrical conductivity of the Lot I seeds was greater than that of the Lot II seeds, regardless of seed number, water volume, and soaking duration (Table 1).

Increasing water volume significantly and consistently reduced the electrical conductivity of Lot I. However, for Lot II, such behavior was not observed when soaking 50 seeds for 48 h or 25 seeds for 72 h, since the average electrical conductivity values for the 50- and 75-mL combinations were statistically similar. In general, increasing seed number from 25 to 50 or 75 did not increase the exudates leaching, except when soaking Lot I seeds for 48 h in 50 or 75 mL distilled water. For example, in 50 mL water, increasing seed number from 25 to 50 or 75 significantly increased electrical conductivity from 13.26 to 16.77 and 16.28 μs cm^{-1} g^{-1}, respectively. In 75 mL water, increasing seed number from 50 to 75 increased electrical conductivity from 7.87 to 10.62 μs cm^{-1} g^{-1}.

The difference observed in the exudate leaching of the lots can be explained by the deterioration of cell membranes in the seeds. The membrane permeability of damaged seeds is greater than that of intact seeds, due to disorganization and low reorganization capacity, which favors solute output during electrical conductivity tests.

According to Menezes et al. (2007), both vigorous, intact seeds and damaged seeds initially exhibit solute leaching, thereby hindering the comparison of lot quality during the initial hours of soaking. According to these authors, the leached solutes liberated by the vigorous seeds is stabilized during the process, due to the membrane reorganization.

The membranes of vigorous seeds may require 48 h to return to their liquid-crystal state, acquiring the semi-permeability, differing from the seeds with low vigor. Afterward, the solute output of the cells to the aqueous solution will be less intense than that observed during the initial hours. However, the heavy exudate leaching by Lot II seeds, alone, might compromise seed quality, due to the loss of fundamental components of metabolism.

Soaking periods of 36 h or more allowed to divide the *Dalbergia nigra* seeds lots (Marques et al., 2002), whereas soaking for 24 h enabled increased the electrical conductivity curves distancing of *Helianthus annuus* lots (Oliveira et al., 2012).

On other hand, studies of *Senna siamea*, *Ricinus communis*, and *Jatropha curcas* were able to distinguish between seed lots during the initial hours of soaking and found that the optimum soaking period was 6 h (Dutra et al., 2007; Souza et al., 2009; Araujo et al., 2011). Similarly, studies of *Solanum sessiliflorum* and *Brassica napus* identified optimum soaking periods of 2 and 8 h, respectively (Pereira and Martins Filho, 2012; Milani et al., 2012), which indicated that the immersion period was more appropriate for assessing seed characteristics than variation in lot vigor.

Increasing soaking duration increased the electrical conductivity of both Lot I and II seeds (Figure 2a). Lot I seeds, which were less vigorous than Lot II seeds, yielded greater mean electrical conductivity values, regardless of soaking duration, with a mean of 19.82 μs cm^{-1} g^{-1}, after 72 h, whereas the mean observed for Lot II seeds after 72 h was 9.35 μs cm^{-1} g^{-1}.

Increasing seed number was also observed to increase electrical conductivity of Lot I seeds, when 50 seeds were used, but did not affect the electrical conductivity of Lot II seeds (Figure 2b). Higher seed mass implies in a higher amount of leached solutes liberated in the soaking solution, accounting only the compounds liberated in the conductivity. However, the similar electrical conductivity values observed for combinations with different numbers of seeds might be explained by dividing the mass of the seeds (μs cm^{-1} g^{-1}), indicating an equivalence on the solute's liberation per gram of fresh mass. In both Lot I and II, increasing water volume gradually reduced electrical conductivity, with means approaching 8 and 5 μs cm^{-1} g^{-1} in the 75-mL combinations, respectively (Figure 2c).

Table 1 - Values of electrical conductivity (µs. cm^{-1}. g^{-1}) of *Melanoxylon brauna* seeds of lots 2010 and 2012 with changes in the quantities of seeds, amount of water and time of soaking.

Distilled water volume (mL)	Lot I			Lot II		
	Seeds quantity					
	25	50	75	25	50	75
	Soaking time: 24 horas					
25	15,428 Ca*	17,833 Cb	15,265 Ca	11,088 Ca	9,974 Ca	11,304 Ca
50	8,978 Bb	9,168 Bb	7,630 Ba	5,356 Ba	6,043 Ba	5,568 Ba
75	8,239 Aa	6,329 Ab	5,112 Aa	3,629 Aa	3,900 Aa	3,476 Aa
	Soaking time: 48 horas					
25	22,505 Ca	32,720 Cc	27,161 Cb	10,786 Ca	13,404 Ca	13,138 Ca
50	13,260 Ba	16,768 Bb	16,275 Bb	8,413 Ba	7,327 Ba	7,741 Ba
75	7,410 Aa	7,872 Aa	10,619 Ab	5,308 Ab	5,007 Bab	4,431 Aa
	Soaking time: 72 horas					
25	32,147 Cb	38,915 Cc	28,577 Ca	12,884 Ca	16,816 Cb	15,747 Cb
50	16,232 Bb	21,392 Bc	10,955 Ba	7,204 Ba	8,118 Ba	7,880 Ba
75	10,682 Ab	11,353 Ab	7,009 Aa	6,282 Ba	4,687 Aa	4,498 Aa

*Means followed by the same uppercase letter un the column, for each imbibition time and seeds quantity, and by the same lowercase letter in the row, for each lot and distilled water volume, do not differ statistically by the Tukey´s test at 5%.

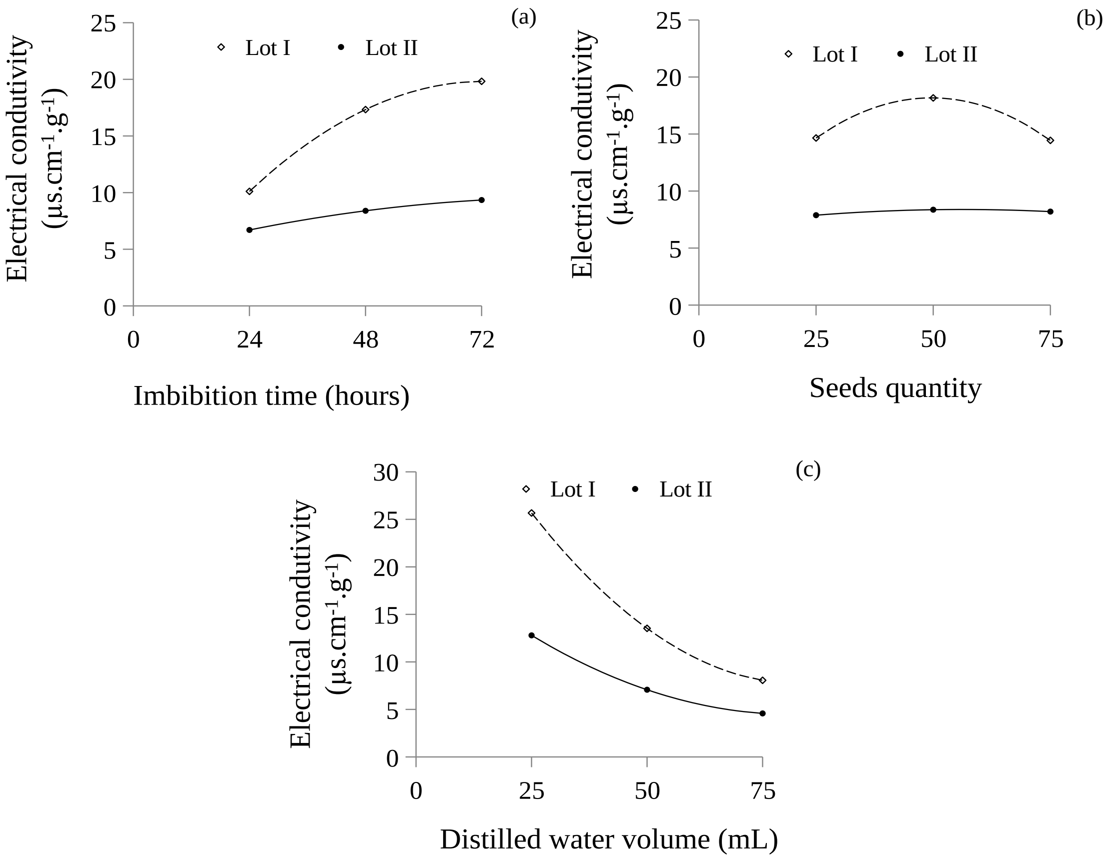

Figure 2 - Electrical Conductivity (µs. cm^{-1}. g^{-1}) of *Melanoxylon brauna* seeds of lots I and II, collected in the years 2010 and 2012, respectively, as a function of the soaking time (A), the number of seeds (B) and volume of water (C).

For forestry species with significant genetic variation in maturation and seed size, authors have reported that increasing seed number improves discrimination between lots, with 75 seeds providing consistent results in *Sebastiania commersoniana* (Santos and Paula, 2005) and *Guazuma ulmifolia* (Gonçalves et al., 2008) and 50 seeds providing consistent results in *Pterogyne nitens* (Ataíde et al., 2012).

When seed mass is held constant, the liquid-crystal structure of hydrated cell membranes allows the passage of a definite quantity of exudates into the water, due to its semi-permeability characteristics. Therefore, greater water volumes yield lower concentrations of liberated solutes, through dilution, and, consequently, yield lower electrical conductivity values. This hypothesis was verified in *Dictyloma vandellianum* seeds, in which 100-mL soaking volumes yielded lower mean electrical conductivity values than 50- and 75-mL volumes (Flavio and Paula, 2010), and in *Solanum melongena* seeds, in which 75-mL soaking volumes yielded lower mean electrical conductivity values than 50-mL volumes (Alves et al., 2012). Accordingly (i.e., due to solute dilution), the 75-mL soaking volume was less efficient in distinguishing between the vigor of the two seed lots. Pereira and Martins Filho (2012) observed a similar pattern, with lower soaking volumes facilitating the discrimination of *Solanum sessiliflorum* seed lots with different qualities, and both Lopes and Franke (2010) and Milani et al. (2012) reported that lower water volumes were more appropriate for measuring the electrical conductivity of *Lolium multiflorum* and *Brassica napus* seeds, respectively. Therefore, there appears to be a direct relationship between the seed vigor differentiation and soaking volume.

Negative correlation coefficients were observed between electrical conductivity test and both germination and GVI, with values ranging from -0.59 to -0.88 (Table 2), and the most significant correlations were observed when soaking 50 seeds in 50 mL for 48 h (r = - 0.86 and - 0.84, respectively; Table 2).

Because the 25-mL soaking volume may hamper the measurement of electrical conductivity, depending on seed size and quantity, 50 mL is recommended as the optimum volume for measuring and comparing the electrical conductivity of *M. brauna* seed lots.

Table 2 - Simple correlation coefficients (r) between the mean values of germination (G) and germination speed index (GSI) and electrical conductivity varying the soaking time, the amount of water and seeds.

Soaking time (hours)	Seeds quantity	Distilled water volume (mL)	Correlation coefficients	
			G	IVG
24	25	25	-0,70*	-0,66*
		50	-0,76*	-0,71*
		75	-0,84*	-0,81*
	50	25	-0,79*	-0,78*
		50	-0,76*	-0,72*
		75	-0,80*	-0,79*
	75	25	-0,63	-0,59
		50	-0,80*	-0,78*
		75	-0,85*	-0,83*
48	25	25	-0,87*	-0,84*
		50	-0,83*	-0,81*
		75	-0,87*	-0,84*
	50	25	-0,87*	-0,85*
		50	-0,86*	-0,84*
		75	-0,80*	-0,79*
	75	25	-0,83*	-0,80*
		50	-0,83*	-0,81*
		75	-0,83*	-0,80*
72	25	25	-0,82*	-0,78*
		50	-0,86*	-0,85*
		75	-0,79*	-0,83*
	50	25	-0,82*	-0,79*
		50	-0,83*	-0,80*
		75	-0,83*	-0,81*
	75	25	-0,82*	-0,79*
		50	-0,88*	-0,86*
		75	-0,87*	-0,85*

* - significant at 5%, by the t-test.

4. Conclusions

Measuring and descriminating between the electrical conductivities of *M. brauna* seed lots is most efficient when soaking 50 seeds in 50 mL distilled water for 48 h quality.

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