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RESEARCH ARTICLE

ELECTRICAL CONDUCTIVITY TEST FOR THE DETERMINATION OF THE PHYSIOLOGICAL SEED QUALITY OF *CROTON FLORIBUNDUS* SPRENG-EUPHORBIACEAE

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ABSTRACT

This experiment studied the efficiency of the electrical conductivity test to evaluate the vigor of *Croton floribundus* seeds. Seven seed lots were evaluated with seedlings emergency and electrical conductivity tests. In the electrical conductivity test, measurements were taken with 25 and 50 seeds samples after 02, 04, 06, 12, 24, 48, 72 and 96 hours of soaking in 75 ml of deionized water at 25 °C. With four replications subjected to analysis of variance in a completely randomized design in split plot where the two quantities of seeds represented the plots and the eight periods of soaking represented the subplots. Data from number of seeds were compared by Tukey test at 5% and for the periods of soaking by polynomial regression. The results indicated that the electrical conductivity test is efficient to separate the lots of capixingui seed and the most effective time period was 96 hours soaking at 75 ml with 25 or 50 seeds at 25 ° C corroborating the results the germination test although they could be already separated with 48 h soaking for 25 seeds samples and 24 hours soaking for 50 seeds samples.

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INTRODUCTION

Since the 1980s, there has been a growing demand for seeds and seedlings of native forest species, which today is driven by the population's awareness of environmental problems and the progress in environmental policy that has led to the increase of restoration projects in order to increase forest areas and climate changes mitigation. Also agroforestry system plantation that had been pointed as very sustainable demands native tree knowledge and good quality seedlings from many species. (Abdo *et al.,* 2008), Those situations have encouraged the use of these species for environmental reforestation, aiming at the recovery of degraded areas, for the production of wood for commercial purposes or for ecological role and biodiversity promotion. This led to an increase in the number of research projects with seeds of native tree species (Santos and Aguiar, 2000). Bonner (1992) points out that the success of these actions requires a consistent technical basis in order to provide viable and good quality seeds for the different objectives of use. According to Piña-Rodrigues (1988), the production of quality seedlings is fundamental, but the establishment of higher forest stands begins with the use of high-quality seeds for the production of seedlings with the desired pattern. In this sense, the use and / or development of seed quality analysis techniques is essential to make seeds with a minimum marketing standard available on the market. Thus, the analysis of the physical, physiological and sanitary parameters of the seeds constitutes what is called seed quality analysis (Cherobini *et al.,* 2008), and can be performed in an isolated way for each of these parameters or more than one. However, the physiological analysis expressed by the germination test and / or vigor tests in general is basic to most seed batch quality characterizations and is very useful for comparing different lots for both sowing and storage (Wielewicki *et al.,* 2006; Gasparin *et al.,* 2012). Research that promotes advances in the technical knowledge on native tree species, contributing to the standardization of germination capacity and vigor tests applicable to the seeds of these species are always very useful. Considering that Brazil has one of the largest floristic reserves in the world, in addition to a great forestry vocation provided by the favorable climatic conditions and the vast territorial extension, studies of this nature are of great importance. *Croton floribundus* Spreng, popularly known as "*capixingui*" in Brazil, is a pioneer tree, belonging to the Euphorbiaceae family, occurring in the States of Rio de Janeiro, São Paulo, Mato Grosso, Minas Gerais and Paraná, mainly in the Semideciduous Broadleaf Forest (Lorenzi, 2002). It exhibits very fast growth and short life cycle, very abundant in secondary formations, occupying gaps and proliferating near forest edges. It is a species widely used in mixed, protective or commercial reforestation, with shading of species of more

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advanced stages of succession (Durigan *et al.,* 2002). This species is typical of the regeneration of riparian forests, as it adapts to the river banks, from the water's edge to the outer edges of the riparian forests, with wide dispersion throughout Brazil. The honey produced from its flowers is almost white in color, with a differentiated aroma, very tasty and of excellent quality. The tree also has medicinal properties (Lorenzi, 2002; Carvalho, 2003). Starts fruiting early, about three years after planting; The fruits are of the tricoca capsule type, containing three seeds, which are rounded, of black color, and that according to Lorenzi (2002) present viability that does not exceed four months. One kilogram contains about 24,000 (Lorenzi, 2002) to 31,000 seeds (Ipef, 2014). Abdo and Paula (2006) testing different temperatures for the germination of *Croton floribundus* seeds recommended as the ideal temperature for conducting the germination test at the alternating temperature of 20-30ºC, with photoperiod of 8 hours. Durigan et al. (2002) recommend the immersion of the seeds in cold water for two hours as a procedure to accelerate and standardize the germination and according to the authors it begins 5 to 10 days after sowing and can extend for up to 90 days (Durigan *et al.,* 2002). However, Abdo and Paula (2006) verified that the germination test for the species can be finished after 28 days. The Rules for Seed Analysis (Brazil, 2009) is a document that proposes the standardization of tests and procedures for seeds of various species, but nothing is said about *Croton floribundus* - Spreng. This confirms what Bonner (1992) has reported that one of the main problems in forest research in tropical regions is the lack of information on flowering phenology, fruit maturation as well as the standardization of the conditions for germination and vigor tests of endemic forest seeds.

Several tests can be used to evaluate the vigor of seed lots, each one has its theoretical basis confirmed and evaluates the quality of the seed lot from some event during the process of deterioration of the seed. In this sense, the electrical conductivity test measures the integrity of the cell membrane system, from the evaluation of the conductivity of the exudates in a solution of imbibition of the seeds. By this test, seed lots with higher values of conductivity, therefore higher electrolyte release in the soaking solution, have the most damaged membrane system and are therefore considered of lower quality and vice versa (Vieira and Krzyzanowski, 1999). In addition to being a good indicator of seed vigor, this test evaluates the initial events of the seed deterioration sequence and has the advantage of being considered a quick, simple and inexpensive test (Torres *et al.,* 1998). The electrical conductivity test has been shown to be efficient in evaluating the vigor of different seed lots of native forest species, corroborating with the results of the germination test and reducing the time of evaluation of seed lot quality of these species. The number of seeds used, the imbibition period and temperature varies from each specie and author as it can be observed in results of some research papers already published.
In this sense some experiment results and the techniques used are described in: Barbedo and Cicero (1998) that recommend for *Inga uruguensis* Hook. & Arn. seeds the use of 20 seeds per replicate soaked in 75 mL of water at 25 ºC for 24 h; Corvello et al. (1999) and Cherobini et al. (2008) recommend for *Cedrela fissilis* Vell. seeds the use of 25 seeds per replicate soaked in 75 mL of water for 24 h, respectively, at 20 ºC and 25 ºC; For *Eugenia pyriniformis* Camb. from Myrtaceae family, Andrade and Ferreira (2000) recommend repetitions of 25 seeds soaked in 75 mL of water at 20 ºC for 24 h; Marques et al. (2002a, 2002b) working with seeds of *Dalbergia nigra* (Vell.) Fr. All. ex. Benth, recommend for repetitions of 50 seeds imbibed in 75 mL of water at 25 ºC for 36 h; Ferreira et al. (2004) recommend replications of 20 seeds soaked in 100 mL of water at 25 ºC for 24 h for *Copaifera langsdorffii* Desf seeds; Santos and Paula (2005) analyzing *Sebastiania commersoniana* (Bail) Smith & Downs seeds from Euphorbiaceae family as the *Croton floribundus* recommend repetitions of 75 seeds soaked in 75 mL of water at 25 ºC for 24 h; Tonin et al. (2005) working with *Pterogyne nitens* Tull seeds used 20 seeds in each replication soaked in 75 mL of water at 20ºC for 24 h although Ataíde at al. (2012) working with the same specie seeds recommended replications with 50 seeds soaked in 50 mL of water for 24 h; Dutra et al. (2007) with S*enna siamea* (Lam.) H.S. Irwin & Barneby recommended 50 seeds soaked in 75 mL of water for 6 h at 30^{o}C; Borba Filho and Perez. (2009) working with *Tabebuia roseo-alba* and *Tabebuia impetiginosa* used 15 seeds with no injuries soaked in 75 mL of water for 24 h at 25^{o}C; Silva et al. (2011) evaluated *Psidium cattleianum* Sabine seeds after storage using four replications of 25 seeds soaked in 75 mL of water at 25^{o}C for 20 and 24 h; Gasparin et al. (2012) working with seeds of *Allophylus edulis* (A. St.-Hil., A. Juss. & Cambess.) Radlk used four replications of 25seeds soaked in 75 mL of water at 25^{o}C for 24 h; Gasparin et al. (2013) also used four replications of 25 seeds soaked in 75 mL of water at 25^{o}C for 24 h for *Parapiptadenia rigida* (Benth.) Brenan1 seeds evaluation; Dalanhol et al. (2014) suggests the use of 25, 50 or 100 seeds replications soaked in 50 mL of water at 25^{o}C during 24 h for studies with *Bowdichia virgilioides* Kunth seeds; Borges et al. (2015) working with *Melanoxylon brauna* Schott seeds used with five replications with 50 seeds / soaked in 70 ml of water at 20ºC for 18 hours.

Considering the absence of information on the quality of seeds of *Croton floribundus* the present work aimed to evaluate the effect of number of seeds per repetition and the period of imbibition of these seeds for conducting the electrical conductivity test, These results with those of the germination test and seedling performance analysis.

MATERIAL AND METHODS

The present work was conducted with seeds of *Croton floribundus* Spreng, called "*capixingui"* in Brazil. The seeds were collected from 25 matrix trees in remnants of the Polo Centro Norte-APTA, in Pindorama municipality, São Paulo State, Brazil. After harvesting the fruits, they were transported to the Experimental Nursery of Ornamental and Forest Plants, Faculty of Agrarian and Veterinary Sciences, Universidade Estadual Paulista (FCAV-UNESP), Jaboticabal *Campus*, where they were placed in the sun under screens with 50% sunlight interception for drying and seed extraction. Once extracted, the seeds were then packed in paper bags and stored in a cool chamber at approximately 10 °C and 60% relative humidity (RH) for approximately 35 days until the initial tests were performed. During this stage of processing, the seeds were kept individually in the bags, separately by each original matrix tree. The seeds lots adopted in this study were constituted by the different procedures performed with seeds or seeds origin matrix tree that resulted in seven different lots: Lot 1- composed of seeds only from matrix 1 with no previous immersion in water; Lot 2- originating from floating seeds, after immersion in water for two hours; Lot 3- originating from seeds that sank (submerged) after immersion in water for two

hours; Lot 4- composed of seeds from some mixed matrices, and which were not immersed in cold water; Lot 5- composed of seeds of matrix 5 with no previous immersion in water; Lot 6- formed by seeds of matrix 8with no previous immersion in water; Lot 7 - consisting of mixed seeds from all trees with no previous immersion in water. Initially, after the formation of the seven seed lots, the water content of each seed was determined by the greenhouse method 105 ± 3 ºC for 24 hours, according to the Rules for Seed Analysis (Brazil, 2009), using two replicates of 25 seeds for each lot. After the determination of the initial water content, the seeds of each lot were submitted to the germination and electrical conductivity test, according to the procedures described below.

Germination test: before the germination test, the seeds of each lot were immersed in cold water for two hours, as recommended by Durigan et al. (2002). Subsequently they were treated with 2% sodium hypochlorite for 10 minutes and then rinsed under running water. The seeds were then placed to germinate in transparent plastic boxes with a lid (gerbox), on a substrate consisting of 20 g of vermiculite of fine granulometry moistened with 30 mL of distilled water, in four replications of 25 seeds. The germination test was conducted in germinators, at the alternating temperature of 20-30 ºC, with photoperiod of 8 hours. The number of germinated seeds was monitored daily, considering the formation of normal seedlings, that is, seedlings with eophilic releases and roots with positive geotropism. The germination test was finished at 28 days, when the germination remained constant. At the end of the test the percentage of normal seedlings and the rate of germination rate were obtained (Maguire, 1962). The experiment was conducted in a completely randomized design with seven treatments (lots) and four replications of 25 seeds. The data were submitted to analysis of variance by the F test and the means were compared by the Tukey test at 5% probability. The percentage data of normal seedlings (G) were transformed into, to meet the assumptions of the analysis of variance.

Electrical Conductivity Test: The electrolyte release pattern of the seeds was evaluated in the seven plots described previously, using two quantities of seeds per replicate (25 and 50 seeds) previously weighed in a precision scale (0.001 g), which were subsequently soaked in 75 mL of deionized water, packed in plastic cups, for different periods (2, 4, 6, 12, 24, 48, 72 and 96 hours) at 25 oC. After each imbibition period, the electrical conductivity of the solution was measured by reading on a Digimed CD-21 conductivity meter, the values of the readings being divided by the sample mass, expressing the results in mS cm -1 g- 1. The electrical conductivity data were submitted to analysis of variance by the F test, according to a completely randomized design, in a 7 x 8 factorial scheme (7 lots and 8 imbibition periods), with four replications, separately by quantity of seeds. The batch averages, for each number of seeds and imbibition period, were compared by the Tukey test at 5% and those referring to the imbibition periods, for each lot and number of seeds, by polynomial regression. In addition, it was calculated the correlation coefficient between germination (G) and germination velocity (VGI) data with electrical conductivity (EC) values.

RESULTS AND DISCUSSION

The water contents of the seed lots of *Croton floribundus* Spreng. (Capixingui) ranged from 7.1 (lot 3) to 9.2% (lot 5), with a variation of 2.1 percentage points, which for some authors would not compromise the results of the electrical conductivity test (Vieira And Krzyzanowski, 1999; Marques, 2002a, 2002b) (Table 1).

Table 1. Water content (%) e variance analysis for germination (G, $arcsen(\sqrt{G/100})$) e Germination Velocity Index (GVI) of seven seeds lots of C*roton floribundus* submitted to germination at 20-30ºC

Variation	Water Content (%)	Freedom degree	Square Mean G	GVI
Lots	-	6	1319,8823**	1,6663**
Residual	-	21	61,7886	0,534
CV%	-		13,78	15,01
Means	-		57	1,54
Lots	-		Means[1]	
1	8,1		71 a	1,53b
2	8,1		34c	0,83c
3	7,1		67 ab	2,31 a
4	8,0		31 c	0,57 c
5	9,2		53 b	1,50b
6	8,2		77 a	2,08 a
7	8,4		67 ab	1,96 ab

Significance levels: * P < 0.05. **P < 0.001. ns = not significant; [1] Means followed by the same letters in each column area not significant by Tukey test at 5%.

Lots 2 and 4 showed the lowest percentages of germination. The highest percentages of germination occurred in lots 6, 1, 3 and 7; Lot 5 presented intermediate germination, but did not differ from the germination of lots 3 and 7 (Table 1). For VGI, the results indicated lots 3, 6 and 7 with higher values, and lots 2 and 4 with lower germination rates. The electrical conductivity test (Table 2) was not efficient to discriminate seed lots of *Croton floribundus* up to 24 h of seed imbibition, with the use of 25 seeds per replicate. However, from 48 h there was discrimination of the seed lots similar to the germination test (G and VGI, Table 1), with small changes in the order of classification of the lots. Using of 50 seeds replications, it was observed that in the shorter periods of imbibition (2, 4 and 6 h) lot 2 differs from the other lots, and in inversed proportional values to the germination test, because in this case lot 2 presented lower conductivity values. A more consistent separation of the lots and corroborating with the results of the germination test occurs only after 48 h of soaking and more accurate after 96 h of soaking with 50 seeds (Table 2), in which the test was more efficient in the separation of the lots according to the quality of the seeds. In this situation, for example, lots 3 and 6 are of better quality than lots 1 and 7, separation not observed by the germination and VGI data; Also, the electrical conductivity test discriminated lot 5 of lots 1 and 7, a fact not observed by the results of germination and VGI. However, the tests were consistent in identifying lots 2 and 4 of lower quality seeds (Tables 1 and 2). The use of the electrical conductivity test is efficient for the separation of lots with similar germination, and the seed imbibition period should be higher when the quality of the lots is close (Vieira and Krzyzanowski, 1999). This fact can be observed in the 24-hour imbibition period which does not differentiate, for example, lots 5 and 7, of lower physiological quality, from lot 6, of better quality. The separation of these lots is only achieved after 48 h of soaking, but more consistently with 96 h with the use of 50 seeds. For Vieira and Krzyzanowski (1999), the period of 24 h would be a recommended average imbibition period. According to the results of Table 2, this period was inefficient for the separation of the lots in the samples of 25 seeds and, although it differentiated some lots in the samples of 50 seeds, this classification was incipient.

Table 2. Mean squares for the water content and electrical conductivity (EC) data of seven lots of *Croton floribundus*, in replications of 25 and 50 seeds, soaked in 2, 4, 6, 12, 24, 48, 72 and 96 hours at 25°C

Variation	Freedom degree			EC - 25 seeds			EC – 50 seeds	
Lots (L)	6			15578,9016**			10730,7765**	
Imbibition period(P)	7			53616,5834			45390,0609	
L x P	42			3576,1850			2282,8650	
Residual	168			602,5315			128,8230	
CV(%)				29,31			14,6871	
Means				83,74			77,27	
Lots				EC – 25 seeds[1]				
	2h	4h	6h	12h	24h	48h	72h	96h
1	45,78 a	52,22 a	57,04 a	64,15 a	72,08 a	80,55 a	94,97 ab	110,11 ab
2	19,76 a	29,13 a	35,16 a	49,39 a	90,44 a	152,77 bc	208,48 e	259,40 d
3	27,48 a	32,29 a	37, 53 a	47,42 a	58,31 a	66,39 a	73,29 a	83,20 a
4	38,04 a	48,84 a	56, 49 a	70,61 a	103,69 a	156,46 c	189,01 de	257,31 c
5	41,48 a	47, 53 a	51,01 a	58,18 a	73,86 a	114,66 abc	151,31 cd	174,37 c
6	41,48 a	49,42 a	53,81 a	57,24 a	63,52 a	72,57 a	80,42 ab	92,36 a
7	54,50 a	64,35 a	68,45 a	76,71 a	94,57 a	102,13 ab	127,46 bc	146,03 bc
Lots				EC – 50 seeds[1]				
1	42,45 b	53,65 b	58,42 b	64,21 a	73,47 abc	86,72 b	99,37 bc	115,56 b
2	15,88 a	24,45 a	32,23 b	45,36 a	80,28 c	129,58 c	170,73 de	207,79 d
3	26,28 ab	30,59 ab	35,24 ab	44,80 a	54,31 a	58,06 a	66,65 a	76,03 a
4	36,13 ab	46,72 ab	54,15 ab	66,79 a	90,30 c	130,74 c	179,41 e	220,53 d
5	38,45 ab	44,41 ab	46,08 ab	52,93 a	77,36 abc	120,45 c	152,34 d	172,62 c
6	37,95 ab	43,39 ab	47,36 ab	52,71 a	58,60 ab	68,29 ab	80,79 ab	91,13 a
7	46,75 b	52,63 b	58,52 b	65,21 a	76,29 abc	91,35 b	109,33 c	125,49 b

Significance levels: * P < 0.05. **P < 0.001. ns = not significant;
[1] Means followed by the same letters in each column area not significant by Tukey test at 5%.

Table 3. Correlation between germination (G) and germination velocity index (GVI) values with electrical conductivity (EC) of *Croton floribundus* seed lots, using 25 and 50 seeds replications, soaked in 75 mL of deionized water, for different periods at 25º C

Seeds	Variables	Imbibition Period (hours)							
mumber		2	4	6	12	24	48	72	96
25	G x EC	0,502^{ns}	0,381^{ns}	0,771*	0,014^{ns}	-0,721^{ns}	-0,958**	-0,942**	-0,965**
	GVI x EC	0,299^{ns}	0,173^{ns}	0,830*	-0,166^{ns}	-0,754*	-0,926**	-0,897**	-0,931**
50	G x EC	0,510^{ns}	0,377^{ns}	0,277^{ns}	0,007^{ns}	-0,778*	-0,891**	-0,927**	-0,945**
	GVI x EC	0,298^{ns}	0,097^{ns}	-0,013^{ns}	-0,259^{ns}	-0,884**	-0,914**	-0,930**	-0,950**

Significance levels: * P < 0.05. **P < 0.001. ns = not significant;
[1] Means followed by the same letters in each column area not significant by Tukey test at 5%.

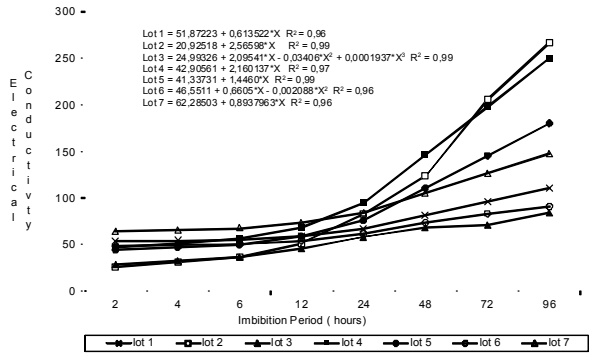

Figure 1. Electrical Conductivity (EC, µS.cm^{-1}.g^{-1}) of lots of *Croton floribundus* Spreng seeds. Replications of 25 seeds soaked in 75 mL of deionized water for different periods of imbibition at 25 ºC

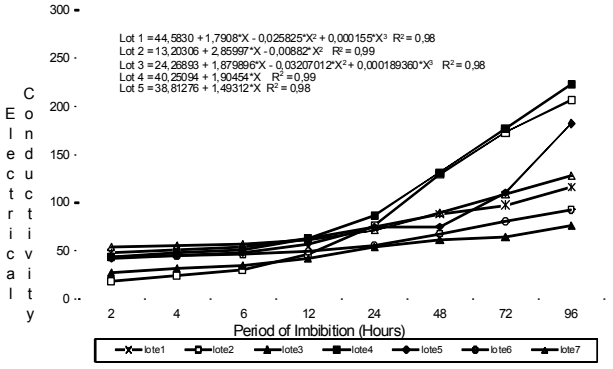

Figure 2. Electrical Conductivity (EC, µS.cm^{-1}.g^{-1}) of lots of *Croton floribundus* Spreng seeds. Replications of 50 seeds soaked in 75 mL of deionized water for different periods of imbibition at 25 ºC

A more similar differentiation to the germination test appears only in the higher periods of imbibition, that is, from 48 h for samples of 50 seeds and from 72 h for 25 seeds. Seed availability may be a factor to consider when choosing the size of the samples and the volume of water used in the installation and conduction of the test. Larger water volumes require larger amounts of seeds, as observed by Santos and Paula (2005), where in experiments with seeds of *Sebastiania commersoniana* (Baill.) Smith & Dows., The authors verified that with 75 mL of water, samples of 75 Seeds had greater efficiency in batch separation than 25 and 50 seed replicates.

The same was observed by Marques et al. (2002b), with seeds of *Dalbergia nigra* (Vell.) Fr. All. Ex Benth., In 75 mL of water, in which samples of 50 seeds and 36 hours of imbibition were more efficient when compared to samples of 25 seeds. Santos and Paula (2005) warn of the fact that a reduced volume of water can lead to problems in the evaporation of the soaking solution and difficulty in reading during the conduction of the test, a fact observed in two plots during the experiment with the seeds from *Croton floribundus*. The leaching pattern of the capixingui seed lots during the imbibition periods, with samples of 25 seeds, highlights lots 3,

6 and 1 of lower electrical conductivity values, while lots 2 and 4 were the highest values of conductivity, and lots 5 and 7 with intermediate values, results that corroborate with those of the germination test (Figure 1, Table 1), with small changes in the order of classification of the lots. For the samples of 50 seeds (Figure 2), the situation is the same where the most vigorous lots present low electrical conductivity values (lots 3, 6 and 1) and those with lower germinative potential present high values of electrical conductivity (lots 2 and 4). However, the use of 50 seeds per replicate gave more homogeneous results within each batch, with lower variability among the replicates, as can be seen from the values of coefficient of variation (CV%) in Table 2, which is of great interest, Because it indicates greater experimental accuracy and more consistent and reliable results. Based on the correlation coefficients between the G, VGI and EC data (Table 3), it was verified that from 48 h of imbibition, regardless of the number of seeds per replicate (25 or 50), there was a high association of Results of the germination test with those of conductivity, with higher correlation values (r> | -0.93) occurring at 96 h of imbibition. Finally, it was verified that the electrical conductivity test was efficient for the separation of seed lots of capixingui and the 96-hour imbibition period in 75 mL was recommended, with 50 seeds at 25ºC, because in this situation, there were The separation of the lots in a more judicious way than the one observed with the germination test, besides the reduction of the time to evaluate the physiological quality of the seed lots.

Conclusion

The electrical conductivity test was efficient to differentiate seed lots of *Croton floribundus* when conducted with 50 seeds per replicate, soaked in 75 mL of water for 96 h at 25 ºC.

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