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Analysis of seed vigor and germination of *Toona ciliata* M. Roem. var. *australis*[1]

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ABSTRACT - Seed analysis tests should be performed under standard conditions for each species, so that the results can be reproducible anywhere. Seeds of the forest species *Toona ciliata* var. *australis* have been commercialized in Brazil despite no standard conditions for their analysis have been published. The objective was to determine basic information about temperature and photoperiod for the germination test and the imbibition time and volume of water for electrical conductivity. In the germination test the seeds were incubated at 15, 20, 25 and 30 °C with 0, 12 and 16 h of light. The electrical conductivity was evaluated up to 108 h after immersion of the seeds in 50 and 100 mL of deionized water. The conditions of 25 °C and 16 h of light provided higher amount of normal seedlings. At 15 °C all seeds remained hard regardless the photoperiod used, however, it became absent when light was added at higher temperatures. The electrical conductivity test should be performed with a volume of 50 mL and evaluated after 24 h of imbibition. It also showed a stronger correlation between normal seedlings, being a promising vigor evaluation test for this species.

Index terms: Australian red cedar, viability, conductivity test, seed quality.

Análise do vigor e da germinação de sementes de *Toona ciliata* M. Roem. var. *australis*

RESUMO - As análises de sementes devem ser realizadas em condições padronizadas para cada espécie, para que assim os resultados possam ser reprodutíveis em qualquer lugar. Sementes da espécie florestal *Toona ciliata* var. *australis* têm sido comercializadas no Brasil apesar de não haver condições padronizadas publicadas para sua análise. O objetivo foi determinar informações básicas sobre temperatura e fotoperíodo para o teste de germinação e tempo de embebição e volume de água para condutividade elétrica. Para o teste de germinação as sementes foram incubadas a 15, 20, 25 e 30 °C com 0, 12 e 16 h de luz. A condutividade elétrica foi avaliada até 108 h após imersão das sementes em 50 e 100 mL de água deionizada. As condições de 25 °C e 16 h de luz proporcionaram maior quantidade de plântulas normais. A 15 °C todas as sementes permaneceram duras independente do fotoperíodo utilizado, entretanto, isso se tornou ausente quando foi adicionado luz em temperaturas maiores. O teste de condutividade elétrica deve ser realizado com volume de 50 mL e avaliado após 24 h de embebição. Esse teste também mostrou uma correlação forte entre plântulas normais, sendo considerado então um promissor teste para avaliar o vigor dessa espécie.

Termos para indexação: cedro-australiano, viabilidade, condutividade elétrica, qualidade de sementes.

Introduction

Seed testing contributes to several research areas, which also brings information to both seed producer and buyer. The germination test, considered as an objective and reproducible for the evaluation of seed quality, is one of the most used tests and universally accepted by several entities (Elias et al., 2012). This test generate economically important information that allow a better understanding of the reproductive strategies of the plant, life history traits and habitat adaptations (Baskin and Baskin, 2014).

Temperature, light and water stress, among other factors, affect the germination process, provoking divergences in the test according to the conditions imposed (Godoi and

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Takaki, 2004; Socolowski and Takaki, 2004; El-Keblawy and Al-Rawai, 2006; Tilki and Dirik, 2007; Yang et al., 2008; Maraghni et al., 2010; Luna et al., 2011; Oliveira et al., 2015). Temperature regulates the seed germination in three ways: determining the capacity and germination rate of non-dormant seeds; removing primary or secondary dormancy; and inducing secondary dormancy. High temperatures can induce secondary dormancy (Bewley et al., 2013) and lead to the death of embryos (Oliveira et al., 2015). Seeds at low temperatures are prone to cooling damages and reduced germination (Fenner and Thompson, 2005).

The light requirement for germination can vary according to temperature, and it is often difficult to separate the effects of light from the temperature (Bonner, 2008; Bewley et al., 2013). According to Bonner (2008), light stimulates the germination of many forest species, being absolutely necessary for just a few. An exposure of just a few minutes, seconds or even milliseconds may be sufficient to start the germination process, while others need intermittent illumination for long periods (Bewley et al., 2013).

Seed testing should follow standardized methodologies so that results can be reproducible among seed laboratories. Regulatory institutions such as Association of Official Seed Analysts (AOSA) and International Seed Testing Association (ISTA) provides standard conditions for substrate, temperature, light, evaluation time, overcoming dormancy methods, and criteria for determination of normal and abnormal seedlings (Elias et al., 2012). In Brazil, seed testing is standardized by the *Coordenação Geral de Apoio Laboratorial* (CGAL) linked to the Ministry of Agriculture, Livestock, and Supply, which publishes the report entitled *Regras para Análise de Sementes* (Rules for Seed Testing), based on ISTA's rules (Brasil, 2009).

The forest species *Toona ciliata* M. Roem. var. *australis* have been widely exploited for having pest-resistant and high-quality wood (Bygrave and Bygrave, 2005). Originally from Australia, it had its first plantations in Brazil around the 70's, in which it found favorable edaphoclimatic conditions for its development (Vilela and Stehling, 2015). It has an advantage in relation to the Brazilian cedars due to its smaller production cycle and the absence of attacks by *Hypsipyla grandella*, and can be used to reduce the overexploitation of native forests, making wood production sustainable (Ferreira et al., 2012).

Besides the studies on the asexual propagation of *T. ciliata* (Souza et al., 2009; Ferreira et al., 2012; Pereira et al., 2015), the production of seedlings in Brazil is based mainly on the commercialization of seeds. The species does not have standardization for the germination tests and therefore the seed quality analysis is can be compromised (Medeiros et al., 2015), bringing consequences for the producer. Based on this, the objective of this study was to determine basic information to subsidized standard conditions for the seed testing of *T. ciliata*, namely: temperature and photoperiod for the germination test and imbibition time and water volume for the electrical conductivity.

Material and Methods

The experiment was carried out at the laboratory of the Department of Horticulture and Forestry belonging to the Faculty of Agronomy of the *Universidade Federal do Rio Grande do Sul* (UFRGS). Although the seeds of *T. ciliata* were purchased from companies accredited in the *Registro Nacional de Sementes e Mudas* (RENASEM) and stored in a cold room at 5 °C until the experiments were installed. Each seed lot was characterized by the state of origin (Santa Catarina - SC, São Paulo - SP and Bahia - BA) and year of collection, being: SC 2014, SP 2014, SP 2015, BA 2014 and BA 2015.

Moisture content

The moisture content of the lots was determined by the oven method at 105 ± 3 °C for 24 h, according to the methodology described by Brasil (2009). Seeds were weighed on analytical balance before and after drying and the moisture content calculated on the basis of initial weight.

Germination test

Seeds were removed from storage, separated from impurities and disinfested in 70% alcohol for 30 seconds and 1% NaClO for 60 seconds, followed by washing in sterilized water for 60 seconds. Afterwards, they were placed on filter paper for superficial drying. Disinfestation was pre-established in a previous study.

They were then distributed in plastic boxes, previously washed with 70% alcohol and 1% NaClO, using blotting paper as substrates (Medeiros et al., 2015) and deionized water. The boxes containing the seeds were packed in plastic bags to prevent loss of water and placed in germinating chambers with temperature and photoperiod regulation. The moisture of the blotter paper was monitored and 2 mL of sterilized water was added when necessary.

The number of germinated seeds was recorded daily for further calculation of the germination rate index (GR) (Maguire, 1962). Based on preliminary studies (data not published), the first germination count was evaluated (FC) after 10 days of incubation counting only the normal seedlings. The germination test ended after 21 days of incubation, recording the number of normal and abnormal seedlings, hard and dead seeds. For the classification of hard or dead,

the seeds were pinched and if interior contents were expel out they were classified as dead, otherwise hard if they remained rigid. Normal seedlings were defined as those possessing the essential structures that are indicative of their ability to produce normal plants under favorable conditions (Elias et al., 2012).

Photoperiod and temperature for germination test

Germination tests were carried out at temperatures of 15, 20, 25 and 30 °C and in photoperiods of 0, 12 and 16 h of light generate by white 20 W fluorescent lamps. For 0 h of light, the plastic boxes were wrapped in aluminum foil in order to avoid any light coming in, and the evaluation of the daily germination and the first germination count were omitted.

In this experiment only the seeds of the BA 2015 lot were used, regarding the higher percentage of germination (unpublished data).

Imbibition curve

The seeds of the BA 2015 lot were removed from storage and weighed in an analytical balance of four decimal places. Afterwards, germination tests were carried out at temperatures of 15, 20, 25 and 30 °C, under a photoperiod of 12 h of light. Soon after incubation, the seeds were weighed at two-hour intervals till complete 12 h of incubation. After that, the weighing was performed at 12-hour intervals, shutting down until 156 h of incubation, or when more than 50% of the seeds had already emitted the radicle. At each weighing, the seeds were removed from the plastic box, dried superficially with filter paper, weighed and put back into the plastic box. For each temperature, four replications containing 25 seeds each were used.

For each weighing interval, the weight gain calculation was performed according to the equation (Albuquerque et al., 2009):

$$WG(\%) = \left(\frac{FW - IW}{IW}\right) \times 100$$

In which: WG = weight gain; FW = final weight of each interval; IW = initial weight prior to imbibition.

Electrical conductivity

The seeds were first weighed into analytical balance, and then inserted into beaker cups containing either 50 mL or 100 mL of deionized water. The cups were sealed with Parafilm M® in order to avoid evaporation of the water and placed in the germinating chamber regulated at 25 °C.

The electrical conductivity of the solution was measured with a Digimed DM-32 conductivity meter after 6, 12, 24, 36, 48, 60, 72, 84, 96 and 108 h of seed imbibition.

Correlation between tests

With the germination test and electrical conductivity data, the Pearson correlation was applied between the variables: number of normal seedlings in the temperature and photoperiod recommended for the germination test, and electrical conductivity of the solution in the time and volume in which there was greater distinction two lots.

Statistical procedures

For the photoperiod and temperature for germination test, the treatments were arranged in a factorial scheme 3 × 4 (photoperiod × temperature), each replication consisting of a plastic box containing 50 seeds. Data were submitted to analysis of variance and polynomial regression. The best fit models were choose based on comparison models using analysis of variance.

For electrical conductivity, all seed lots were used and the treatments were arranged in factorial scheme 2 × 5 × 10 (water volume × lot × imbibition seed time). The data were submitted to analysis of variance, with each replication composed of a beaker containing 25 seeds. Within each imbibition time and volume of water, the electrical conductivity averages were compared by the Tukey test at 5%.

The design was completely randomized in all experiments, each treatment being composed of four replications. Data were submitted to the Shapiro-Wilk normality test and the Bartlett variance homogeneity test. All analyzes and graphs were performed using RStudio software (version 1.0.136).

Results and Discussion

The SP 2014, BA 2014 and BA 2015 lots presented an initial moisture content lower than 10% (Table 1), which is adequate to maintain viability during the storage of orthodox seed (Bonner, 2008; Karrfalt, 2008). On the other hand, the SC 2014 and SP 2015 lots contained an average of 13.09% of moisture content.

Table 1. Initial moisture content of *Toona ciliata* var. *australis* seed lots marketed in Brazil.

Lots	Moisture content (%)
SC 2014	12.9 a
SP 2014	6.9 c
SP 2015	13.2 a
BA 2014	9.8 b
BA 2015	9.9 b

In which: SC 2014 = Santa Catarina lot collected in 2014; SP 2014 = São Paulo lot collected in 2014; SP 2015 = São Paulo lot collected in 2015; BA 2014 = Bahia lot collected in 2014; BA 2015 = Bahia lot collected in 2015. Means followed by the same letters in the column do not differ statistically by the Tukey test at 5%.

Photoperiod and temperature for germination test

The GR variable showed a quadratic behavior with maximum value at 28 °C, where the germination occurred in less time (Figure 1 - A). Similar results were observed for the FC variable, but only for the seeds submitted to the 16 h of light (Figure 1 - B). Using 12 h of light the response was linear, showing 47.55% of germinated seeds at 30 °C.

These results corroborate with those of other works related to the germination of tropical forest species, like *Tecoma stans* L. Juss. ex Kunth (Socolowski et al., 2008), *Caesalpinia ferrea* Mart. Ex Tul. (Melo et al., 2017), *Euterpe precatoria* Mart. (Costa et al., 2018), and several others Brazilian native species (Brancalion et al., 2010).

T. ciliata showed rapid germination at temperatures of

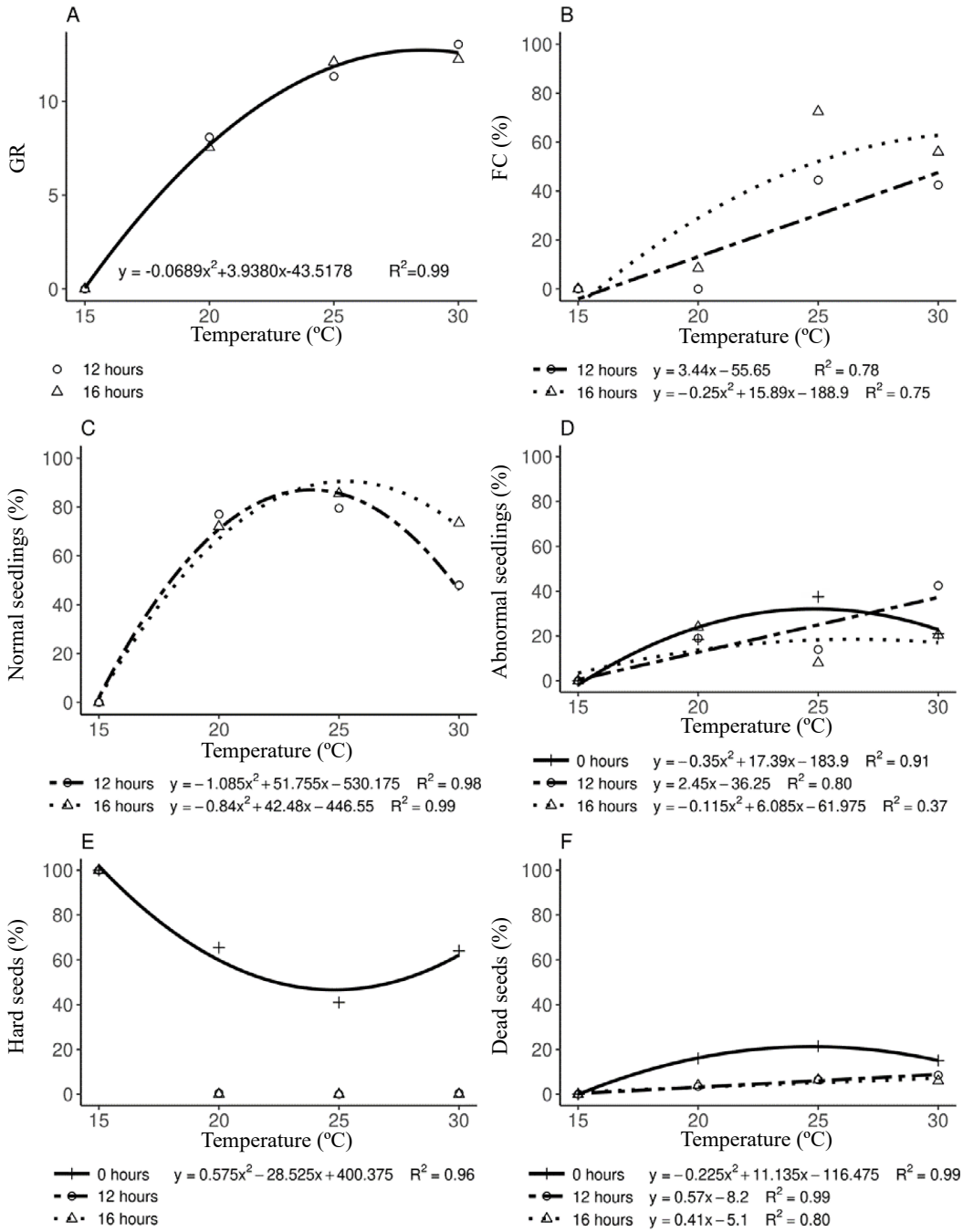

Figure 1. Germination test of *Toona ciliata* var. *australis* seeds under different photoperiod and temperatures. A – GR (germination rate); B – FC (first count); C – Normal seedlings; D – Abnormal seedlings; E – Hard seeds; F – Dead seeds.

25 °C with 16 h of light, in which more than 50% of their seeds had already germinated after 10 days of incubation (FC). Among the timber species of Australia, the germination of *T. ciliata* is classified as moderately rapid (Smith et al., 2008), corroborating with the data found in the present work.

Regarding normal seedlings, the data analysis showed a negative quadratic behavior for 12 and 16 h of light (Figure 1 - C). At 25 °C and 16 h of light the seeds generate 90.52% of normal seedlings, higher than that found for the 12 h of light, 87.01% at 24 °C. At 12 h the seedlings did not handle the temperature effects and showed signs of wilt. This was not observed with 16 h of light, which could have helped the seeds overcome the deleterious effects caused by the high temperature. With 0 h of light all seedlings etiolated and were classified as abnormal seedlings. It was also observed that temperature of 30 °C generate a greater percentage of abnormal seedlings in the 12 h of light, a 20% increase compared to 16 h (Figure 1 – D). The higher temperature tolerance with 16 h of light may have occurred due to the enhanced activity of leaf aquaporins. According to Maurel et al. (2008), the enhanced activity of leaf aquaporins may favor water transport into the inner leaf tissues when transpiration is maximal, avoiding damage to the plant. In the total absence of light, the maximum value for abnormal seedlings was similar to that found for normal seedlings, which was at 25 °C.

The value of normal seedlings generated at 25 °C and 16 h of light was higher to that found in other works with *T. ciliata* (Smith et al., 2008; Medeiros et al., 2015; Migliorini et al., 2015), demonstrating, therefore, that these conditions are ideal for germination tests with this species, since they provide low interferences in the viability of the seeds. Regardless of light hours, all seeds submitted to a temperature of 15 °C did not germinate but remained hard until the final evaluation. The increase in temperature reduced the amount of hard seeds, being null in the 12 and 16 h of light with temperature between 20 and 30 °C. In the absence of light, there was also a reduction of hard seeds, with a negative quadratic behavior in which the minimum value at 25 °C was 46.60%, higher than seeds at 12 and 16 h (0%).

This work showed the effect of the photoperiod and temperature interaction on seed germination ($p<0,001$), since the data demonstrate that the temperature increase provided stimuli for germination, but it was not enough for the seeds submitted to absence of light. Low germination in the absence of light was also observed in *Acacia polyphylla* DC. seeds, in which the use of only 1 h of daily light was sufficient to double the amount of germinated seeds (Araújo-Neto et al., 2003).

Imbibition curve

The first 12 h of incubation were characterized by a rapid imbibition of water, in which the weight gain in relation to the initial mass reached values above 100%, regardless of temperature (Figure 2). Then, the seeds weight remained stable for a different period at each temperature (96 h at 20 °C and 36 h at 25 and 30 °C). With the emission of the radicle, another increase in weight gain was observed, rising gradually as more seeds emitted the radicle. This experiment showed the three-phase pattern of the *T. ciliata* germination, in which Phase I is characterized by a rapid imbibition of water, Phase II by weight stabilization, and Phase III by increasing weight gain after stabilization.

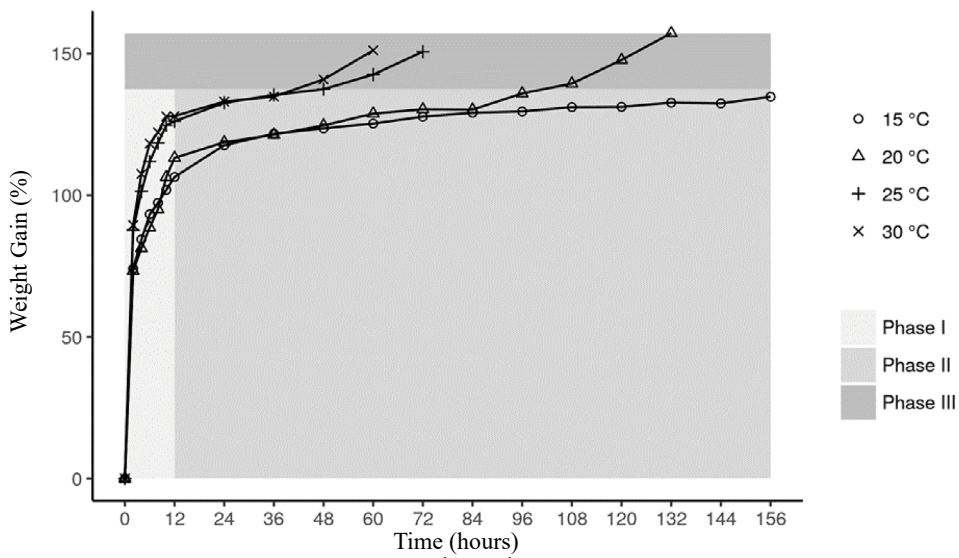

Figure 2. Imbibition curve of *Toona ciliata* var. *australis* seeds under 12 h of photoperiod and different temperatures.

Phase I occurs in seeds with physiological dormancy, not dormant, viable or dead, as it is a consequence of the matrix forces of the osmotic gradient between seed and water, that is, it is an essentially physical process (Bewley et al., 2013). Some cases may hinder the absorption of water at this stage, such as the presence of hard integument in the seed forming a barrier to water entry (Dübbern-Souza and Marcos-Filho, 2001). The *T. ciliata* seeds do not possess this property, allowing a rapid imbibition soon after contact with water. At the end of Phase I the seeds submitted to temperatures of 15 and 20 °C absorbed less water than at 25 and 30 °C.

A greater prolongation of the Phase II was observed at 15 °C, corroborating with the data obtained in the germination test at the same temperature, resulting in 100% of hard seeds at the end of the test (21 days). Phase II is affected by the osmotic potential of the water sources for the seed and the environment temperature, having a longer duration with lower the temperature due to the low respiratory rate of the embryo (Bewley et al., 2013).

Electrical conductivity

The results showed differences between the lots at all times and volumes tested, but with different group formations between each time and volume (Table 2). Lot separation was more sensitive in 24-hour soak to the 50-mL volume, and in 60-hour soak to 100 mL, at which points the group by the means test was identical. To facilitate the execution of the test, it could be indicated the time of 24 h and 50 mL of volume of water to perform the electrical conductivity test for *T. ciliata*. The BA 2015 lot presented the lowest electrical conductivity among the lots tested, indicating a greater vigor in relation to the others.

When water is soaked, cell membranes reorganize and repair the damage from storage, but until the repair is complete, several cell solutes are leached into the medium (Schmidt, 2007). The increase of the imbibition time allowed a greater quantity of solutes to be leached, a fact also observed for other forest species like *Dalbergia nigra* (Vell.) Fr. All. ex Benth. (Marques et al., 2002), *Sebastiania commersoniana* (Bail) Smith & Downs (Santos and Paula, 2005) and *Dictyoloma vendellianum* A. Juss (Flavio and Paula, 2010).

The use of more seeds could reduce the amount of soaking required for lot separation, as demonstrated by Santos and Paula (2005), in which the use of 25% more seeds decreased in 16 h the time to separate lots of *Sebastiania commersoniana* (Bail) Smith & Downs seeds. The electrical conductivity test was also efficient for the lots separation of *Dalbergia nigra* (Vell.) Fr.All. ex Benth., in which the use of 30 or 36 h for the imbibition of the seeds is recommended (Marques et al., 2002).

Correlation between tests

All tests showed a higher quality of the BA 2015 lot, which was classified as high viability and vigor. There was also a significant correlation between the amount of germination test normal seedlings at 25 °C and 16 h of light and the electrical conductivity after 24 h of imbibition in 50 mL (-0.8386 **), showing that lots with low electrical conductivity can germinate more normal seedlings. The electrical conductivity test yields satisfactory results for the viability assessment and can be used as a complementary test to the germination test.

According to Elias et al. (2012) the electrical conductivity test is not effective for all crops, but the correlation between

Table 2. Electrical conductivity (μS $cm^{-1}.mg^{-1}$) of *Toona ciliata* var. *australis* seed lots marketed in Brazil, with different volumes and imbibition period.

Lots	50 mL									
	6 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h
SC 2014	328.7 a	347.1 a	366.5 b	429.7 a	477.5 ab	521.1 ab	569.4 ab	591.5 ab	619.9 ab	634.3 ab
SP 2014	343.9 a	364.4 a	440.5 a	498.5 a	526.1 a	582.9 a	654.7 a	695.5 a	740.1 a	748.6 a
SP 2015	296.5 ab	321.9 ab	373.6 b	428.1 a	445.6 b	471.7 b	507.2 b	529.2 b	564.3 b	576.2 b
BA 2014	248.7 b	260.9 b	246.8 c	265.8 b	265.3 c	278.8 c	290.2 c	295.1 c	299.0 c	306.8 c
BA 2015	166.3 c	179.3 c	170.5 d	180.9 b	181.7 d	188.2 c	208.1 c	215.0 c	223.2 c	223.6 c
Lots	100 mL									
	6 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h
SC 2014	175.8 ab	181.3 ab	202.0 b	224.0 ab	224.0 ab	240.0 b	257.5 ab	260.6 ab	278.1 ab	298.4 a
SP 2014	188.8 a	205.6 a	233.0 a	246.4 a	246.4 a	274.6 a	289.6 a	301.4 a	319.0 a	316.2 a
SP 2015	145.1 b	161.2 b	192.2 b	200.9 b	200.9 b	220.6 b	238.2 b	244.8 bc	256.5 b	261.3 ab
BA 2014	157.5 ab	169.7 b	164.7 c	163.9 c	163.9 c	180.0 c	190.7 c	197.4 c	199.2 c	201.9 b
BA 2015	81.7 c	86.54 c	86.74 d	89.06 d	89.06 d	96.94 d	103.1 d	106.1 d	115.0 d	119.0 c

In which: SC 2014 = Santa Catarina lot collected in 2014; SP 2014 = São Paulo lot collected in 2014; SP 2015 = São Paulo lot collected in 2015; BA 2014 = Bahia lot collected in 2014; BA 2015 = Bahia lot collected in 2015. Means followed by the same letters in the column do not differ statistically by the Tukey test at 5%; ^{ns} = not significant.

these test and the germination test was also observed in other forest species, as in *Jacaranda micrantha* Cham. (Souza et al., 2016), *Dalbergia nigra* (Vell.) Fr.All. ex Benth. (Marques et al., 2002) and *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S. Johnson (Gonzale et al., 2011). Therefore, the use of these tests has shown to be promising in the evaluation of vigor in forest seeds, reducing the time necessary to classify lots according to the physiological quality.

Conclusions

For germination tests of *Toona ciliata* var. *australis* it is recommended to incubate the seeds in 16 h of light with temperature of 25 °C.

The electrical conductivity test should be performed with a volume of 50 mL and evaluated after 24 h of imbibition.

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