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Basic procedure for the *in vitro* propagation of Brazilian trees for reforestation purposes

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ABSTRACT

Important strategies can be used to avoid biodiversity loss by deforestation in tropical rainforests. Some use biotechnological techniques to support conservation initiatives. Plant tissue culture techniques are highly accepted biotechnological approaches for conservation of biodiversity. The work aimed to propose a basic operational model for the induction of *in vitro* germination of trees through plant tissue cultivation techniques. Fruits of 15 tree species, ten woody trees (*Couroupita guianensis* Aubl., *Tabebuia heptaphylla* (Vell.) Toledo, *Tabebuia impetiginosa* (Mart. ex DC.) Standl., *Tabebuia roseoalba* (Ridl.) Sandwith, *Vochysia haenkeana* (Spreng.) Mart., *Vitex montevidensis* Cham., *Copaifera coriacea* Mart., *Spondias tuberosa* Arruda, *Schinus terebinthifolia* Raddi, and *Talisia esculenta* (A. St.-Hil.) Radlk.) and five palm trees (*Syagrus coronate* (Mart.) Becc., *Attalea oleifera* Barb. Rodr., *Elaeis guineensis* Jacq., *Colubrina glandulosa* Perk., and *Astrocaryum vulgare* Mart.) were collected at different locations in the State of Pernambuco, Brazil. The *in vitro* germination used two different protocols, one designed for palm trees and one designed for woody trees. It was evaluated the parameters microbial contamination, survival, *in vitro* establishment, germination percentage and percentage of seeds converted to plants. The results showed that the set of methodologies proposed as a basic protocol for the *in vitro* introduction was able to achieve satisfactory results for 13 of the 15 tested species. The protocol proposed a high potential for use in the rescue of seeds through *in vitro* plant tissue culture. The described technique is an efficient tool for the propagation of trees used in reforestation programs.

Keywords: Plant tissue culture, woody trees, palm trees.

Introduction

The loss of genetic variability through deforestation and other human activities is part of the factors that severely affect various biomes in the world. Therefore, conservation investments are strategically important to minimize the level of genetic diversity loss. Conservation programs need to be applied quickly to reduce species extinction in some important biomes; this has become more evident in global endemic areas. Global Biodiversity Hotspots (GBHs), which account for almost 50% of endemic plant species, have received particular attention in recent years. Biotechnological strategies for plant conservation can be used to reduce mass extinction of species. The seed recovery and *in vitro* germination for the conservation of germplasm can be a strategic tool with the potential application to minimize the severe losses in biomes such as the Atlantic Forest (which now only covers less than 5% of its original area). The *in vitro* cultivation of plants has already been successfully used for the production of various tree species from different ecosystems (Oliveira et al., 2013). In this case,

seeds of endangered species with short flowering and fruiting periods are collected and used to grow seedlings throughout the year; seedlings produced from different strains of seeds can be used in reforestation and forest enrichment programs.

The uses of tissue culture techniques for woody plant conservation purposes have already been described and are well known within the scientific community (Theilade & Petri, 2003; FAO, 1993). In fact, tissue culture is considered a valuable alternative for endangered plant species conservation (Sarasan, 2010). However, the *in vitro* establishment protocols and subsequent production of viable plants can vary greatly between different species (Ram et al., 2012; Pasqual et al., 2012; Parihar & Kumar, 2015; Vengadesan & Pijut, 2009). This difference in methodology is one of the main factors that interfere with the adoption of plant tissue cultures to propagate different woody plant species simultaneously. Nevertheless, the use of plant tissue cultures can be a valuable tool to obtain seedlings for the reforestation of disturbed areas as well as to manage endangered species.

The aim of the present work is to establish a basic disinfestation and *in vitro* germination protocol for different species of woody plants endemic to Brazil to develop a basic methodological set for the early stages of woody plant establishment.

Material and Methods

This study was conducted through collection and survey from January 2014 to January 2015 at the Laboratório de Pesquisas Aplicadas à Biofábrica (LAPAB), which is a part of the Centro de Tecnologias Estratégicas do Nordeste (CETENE) in Recife, Pernambuco. The methods proposed are based on the use of seeds as explants of different woody species; the seeds originated from ripe and intact fruit and were collected from matrices with apparent vigor. To generate a basic operational model that could be applied to different kinds of woody plants, we established two primary steps in the *in vitro* introduction process. The first step is the establishment of the method for disinfestation from the collected plant material to reduce microbial contamination; the second step is a standardization of the seed germination method. We evaluated 15 different species; 10 woody trees and five palm trees. All elected species are endemic to Brazilian biomes, three of them are already cataloged in the Red List of endangered species of the IUCN (International Union for Conservation of Nature) (Table 1).

The fruits collected of the 15 species (Table 1) were washed under running water with commercial neutral detergent with the help of a soft sponge for 5 min. Subsequently, in a laminar flow cabinet, they were immersed in 70% ethanol solution (v:v) for 2 min and then in commercial sodium hypochlorite solution (2.5%) for 10 min; after rinsing three times with autoclaved distilled water, the fruits were opened and the seeds separated from the pulp.

For the *in vitro* establishment of seeds, we used two types of MS media (Murashige & Skoog, 1962) for woody and Y3 medium (Eeuwens, 1976) for palm trees. The MS medium was composed of MS macro- and standard micronutrients plus agar (6.0 $g.L^{-1}$), sucrose (30.0 $g.L^{-1}$). The pH was adjusted to 5.8. After addition of the agar, 30 mL of each medium were distributed in 50 mL test tubes and autoclaved (121°C for 20 min). Depending on the size of the seeds, inoculations were performed in test tubes (10 mL medium/tube) or flasks (30 mL medium/tube). After inoculation of about 90 seeds per species in the culture medium, the explants were kept in a growth room with light control (40 $\mu mol.m^{-2}.s^{-1}$) at a temperature of 25 ± 2°C with a 16 h light photoperiod. The number of seeds inoculated in the culture medium varied according to the specific type of fruit and its availability (Table 1). It was evaluated germination rates, contamination, and oxidation. Data analysis was performed using averages and percentages.

Table 1. Characterisation of species evaluated to establish the protocol of *in vitro* introduction (Souza, 2017).

Scientific name	Brazilian common name	IUCN Red List	Tree type	Biome
Astrocaryum vulgare Mart.	Tucumã	NA	Palm	CE
Tabebuia impetiginosa (Mart. ex DC.) Standl.	Ipê roxo	Lower risk (1998)	Woody	CE/CA
Tabebuia roseoalba (Ridl.) Sandwith	Ipê branco	NA	Woody	CE/CA/MA
Tabebuia heptaphylla (Vell.) Toledo	Ipê rosa	NA	Woody	MA
Syagrus coronate (Mart.) Becc.	Licuri	NA	Palm	CA
Attalea oleifera Barb. Rodr.	Pindoba	Lower risk (1998)	Palm	MA
Elaeis guineensis Jacq.	Dendê	NA	Palm	MA

Colubrina glandulosa Perk.	Suruají	NA	Palm	CE/MA
Vochysia haenkeana Mart.	Pau-Mulato	NA	Palm	CE
Copaifera coriacea Mart.	Sapucaia	NA	Woody	CA/PT
Couroupita guianensis Aubl.	Abricó de macaco	Lower risk (1998)	Woody	AM
Vitex montevidensis Cham.	Tarumã	NA	Woody	MA/PP
Spondias tuberosa Arruda	Umbu	NA	Woody	CA
Schinus terebinthifolia Raddi	Aroeira	NA	Woody	CA
Talisia esculenta (A. St.-Hil.) Radlk.	Pitomba	NA	Woody	CE

NA=Taxon which has not yet been assessed for the IUCN Red List; CA=Caatinga; CE=Cerrado; MA=Mata Atlântica; AM=Amazônia; PP=Pampa; PT=Pantanal.

Results

In vitro contamination control is a basic requirement for tissue culture technology establishment. In the present study, the percentage contamination of the explants (seeds) during the *in vitro* establishment showed that each species has specific endophytic microorganisms, and the success of *in vitro* establishment can vary depending on the natural condition of plant seeds. The highest survival percentage (above 50%) was observed for 10 species, three palm trees and seven woody trees (Table 2). Therefore, the use of one standard disinfestation protocol, which consisted of washing the fruit followed by disinfestation in 70% alcohol and 2.5% sodium hypochlorite, was effective for 90% of the woody tree and palm species.

Seed germination potential is also an important factor for *in vitro* protocol establishments. Many tropical plants have recalcitrant seeds. In this study, it was observed that the palm tree species with the highest percentage of *in vitro* germination had the lowest percentage of conversion into adult plants. In contrast, seed germination of woody tree species ranged from 0 to 100%, with no correlation between the percentage of germination and the success rate in plant conversion (Table 2).

Among the palm trees, *Astrocaryum vulgare* presented the lowest germination rate (7%), which was considerably lower than germination rates observed for other palm species (*Attalea oleifera*, *Elaeis guianeensis*, and *Colubrina glandulosa*). However, all *A. vulgare* seeds that germinated formed plants *in vitro*. Considering other palm trees (*A. oleifera*, *Syagrus coronate,* and *E. guianeensis*), plant development rates varied between 15 and 70% (Table 2).

Table 2. Percentage of seed germination under *in vitro* culture conditions and the effect on plant development (Souza, 2017).

Species	**Brazilian common name**	***In vitro* germination (%)**	***Plants from germinated seeds (%)**
Astrocaryum vulgare	Tucumã	7.0	100
Tabebuia impetiginosa	Ipê roxo	60.0	90
Tabebuia roseoalba	Ipê branco	25.0	60
Tabebuia heptaphylla	Ipê rosa	40.0	95
Syagrus coronate	Licuri	80.0	40
Attalea oleifera	Pindoba	65.0	40
Elaeis guineensis	Dendê	60.0	15
Colubrina glandulosa	Suruají	70.0	70
Vochysia haenkeana	Pau-Mulato	0.0	0.0
Copaifera coriacea	Sapucaia	45.0	100
Couroupita guianensis	Abricó de macaco	90.0	100
Vitex montevidensis	Tarumã	0.0	0.0
Spondias tuberosa	Umbu	100.0	80
Schinus terebinthifolia	Aroeira	80.0	100
Talisia esculenta	Pitomba	100.0	100

* Number of seedlings which developed epicotyl and root system after germination.

On woody trees, there was a clearer variation of *in vitro* viability. Two species did not germinate (*Vitex montevidensis* and *Vochysia haenkeana* (Spreng.) Mart.; other eight species showed the viability of seeds, all wing the *in vitro* establishment and subsequent formation of plants (Figure 1).

Figure 1. Germination and *in vitro* development of different species of forest trees. Bar = 1 cm (Souza, 2017).

The results observed for 13 of 15 species studied indicate the ease and feasibility of the use of *in vitro* seed cultivation techniques for the propagation of forest species. As such, the most successful strategy for the simultaneous cultivation of different species requires rapid *in vitro* introduction after fruit collecting.

In vitro germination of some target species started after 15 days, as seen in *A. oleifera*, taking up to 60 days for *C. glandulosa*. The period required to break seed dormancy and start *in vitro* development varied between species. In general, the lack of growth regulators during *in vitro* establishment phase for the analyzed variables did not limit initial seed development. However, the use of low concentrations of BAP (6-Benzylaminopurine) in the cultivation process may enhance the development of the explants in subsequent steps.

These results indicate that germination can be improved by some modification of the process. However, using just one protocol for disinfestation and seed *in vitro* germination is suitable for the simultaneous cultivation of different tree species.

Regardless of the time, the initiation of the *in vitro* germination is a good indicator of the applicability of tissue culture for the production of seedlings of forest tree species.

Discussion

The results suggest that the methodology used to collect explants, decontamination and *in vitro* introduction were adequate for most of the species studied and can be used routinely; it may also be a valid procedure for the propagation of other tree species. According to Grattapaglia & Machado (1998), establishing an aseptic culture is the most critical phase of *in vitro* cultivation. Thus, the success of the micropropagation technique is based on the recommendation of an asepsis protocol and *in vitro* establishment to achieve the greatest number of aseptic explants *in vitro*, reducing the production of phenolic compounds (oxidation) and converting explants into initial plans for the next stages of seedling production.

In some cases, the use of 70% alcohol and sodium hypochlorite is not sufficient to control contamination of the plant tissues with microorganisms. According to Butt et al. (2013), the traditional disinfestation protocol using bleach (commercial sodium hypochlorite solution) was not effective to overcome guava explant contamination. In this case, to control the high level of fungal and bacterial contamination, the authors used bleach and a strong acid solution (10% HCl for 24-72 h), thereby successfully reducing guava contamination rates from 98 to 0%. However, many plant species can be successfully disinfected by using only alcohol (70%) and sodium hypochlorite solution (2%).

Sodium hypochlorite (NaOCl) has been widely used to successfully control bacteria and fungi on plant tissue surfaces (Emmanuel et al., 2004), for example in seeds of *Rhodiola rosea* L. (Tasheva & Kosturkova, 2012), *Syngonanthus elegantulus* Ruhland (Pêgo et al., 2013) and

Byrsonima intermedia A. Juss. (Silva et al., 2015). The antimicrobial activity of NaOCl is attributed to the hypochlorous acid (HOCl) which can penetrate the microorganism cell wall (Len et al., 2002); NaCl is therefore routinely used in the majority disinfestation protocols for plant tissue cultures.

The lack of knowledge about the physiological processes of many species, especially woody plants, has hindered reforestation programs by *in vitro* cultivation. Thus, to establish a "basic operational model", procedures for *in vitro* establishment of woody species to optimize projects to support the spread of different species to promote biome conservation are needed.

Microorganisms are one of the main factors that can interfere with seed germination (Martins-Corder & Borges Junior, 1999). According to Couto et al. (2004), tree species are particularly difficult to establish *in vitro* due to the diversity of contaminating microorganisms.

In this study, the observed endophytic contamination (fungi, bacteria, and yeasts) did not completely impede the development of *in vitro* plants (Table 3). Thus, despite the diversity and contamination percentage observed in different species of woody trees, in extreme cases in *Tabebuia heptaphylla* and *E. guianeensis* Jacq. this method can be used for *in vitro* germination of seeds, providing the conservation of part of the genetic variability generated by mother plants (matrices).

Similar results have previously been described in the literature, even with the use of different procedures and substances than the ones evaluated in this study. In some cases, according to Nascimento et al. (2007), efficient disinfection of seeds has been achieved by applying calcium or sodium hypochlorite with fungicides and bactericides, thereby maximizing the percentage of germinated seedlings. However, in most cases, using a combination of alcohol and sodium hypochlorite was sufficient to achieve effective levels of seeds disinfection.

In mahogany (*Swietenia macrophylla* King) seed disinfestation studies, Couto et al. (2004) observed 89% contamination when the seeds were not treated with disinfecting substances; other researchers disinfected mahogany seeds with 70% ethanol and 5.5% commercial sodium hypochlorite (Valverde-Cerdas et al., 1998). In the case of *Cedrela fissilis* Vellozo, the seeds were sterilized with sodium hypochlorite at 2.5% (Nunes et al., 2002). Contamination after disinfestation may be due to the existence of endophytic microbial colonies which cannot be reached by the disinfection substances.

Table 3. Percentage of contamination by fungi and bacteria, and the percentage of survival of seeds under *in vitro* culture conditions (Souza, 2017).

Species	**Brazilian common name**	****Fungi**	****Bacteria**	***Survival**	***In vitro* establishment**
Astrocaryum vulgare	Tucumã	7.0	0.0	7.0	Yes
Tabebuia impetiginosa	Ipê roxo	11.0	5.5	72.0	Yes
Tabebuia roseoalba	Ipê branco	0.0	6.2	34.0	Yes
Tabebuia heptaphylla	Ipê rosa	21.5	2.0	46.0	Yes
Syagrus coronate	Licuri	20.5	0.8	90.0	Yes
Attalea oleifera	Pindoba	16.0	10.0	85.0	Yes
Elaeis guineensis	Dendê	11.0	13.0	94.0	Yes
Colubrina glandulosa	Suruají	6.0	0.7	79.0	Yes
Vochysia haenkeana	Pau-Mulato	92.0	8.0	0.0	No
Copaifera coriacea	Sapucaia	3.5	2.5	100.0	Yes
Couroupita guianensis	Abricó de macaco	2.5	0.0	100.0	Yes
Vitex montevidensis	Tarumã	0.0	0.0	0.0	No
Spondias tuberosa	Umbu	0.0	25.0	100.0	Yes
Schinus terebinthifolia	Aroeira	5.0	0.0	100.0	Yes
Talisia esculenta	Pitomba	0.0	0.0	100.0	Yes

*Percentage of seeds that developed into plants from explants uncontaminated *in vitro*. ** Percentage of fungal and bacterial contamination.

As evidenced by the results showed in the Tables 1 and 2, the two species that were not viable (*Vitex montevidensis* and *V. haenkeana*) presented the association of two problems: endophytic contamination associated with low seed viability.

The results regarding the success of germination percentage and conversion

percentage in plants (Table 1 and 2) shows that, despite optimization, intrinsic characteristics of each species will determine the process of the potential success and the following inclusion of the species in multiplication routine for the purpose of *in vivo* reintroduction.

According to Berjak & Pammenter (2000), analyses of several recalcitrant seeds of gymnosperms, dicots, and monocots indicated that potential germination maintenance is essential to seed development. This study showed a relation between seed development/germination potential and water loss tolerance. Therefore, the differences among seeds of different plant species are associated with cell ultrastructure and water storage reserve maintenance; the best strategy for the simultaneous cultivation of different species thus requires a rapid introduction *in vitro* after fruit collection.

Despite the various time requirements for germination, *in vitro* culture can facilitate seed dormancy breakage. According to Yang et al. (2013), light and temperature can positively influence seed germination. However, pH and some phytoregulators have no effect or even damage the subsequent development phases. In contrast, for *Grewia tenax* (Forssk.) Fiori, the seed-coat could be more important than *in vitro* conditions for seed germination (Daffalla et al., 2016).

In studies on *in vitro* germination of *Amburana acreana* (Ducke) A.C. Sm., Fermino-Junior et al. (2007) observed the occurrence of *in vitro* germination after eight days of inoculation. Similar results have been described by Lopes (2000) and Lemos et al. (1998) studying mahogany, where germination was initiated in six 10-day periods, while other species, such as pau rosa (*Aniba roseodora* Ducke) and sucupira rosa (*Pterodon pubescens* (Benth.) Benth.) are not described as having good *in vitro* germination and need more time to germinate (França et al., 1997). According to Fay (1992), for some species, seed germination increases with the use of tissue culture techniques.

In the propagation of woody tree species such as paricá (*Schizolobium amazonicum* Huber ex Ducke), eucalyptus (*Eucalyptus globulus* Labill.), and sucupira branca, the use of growth regulators has shown likely impacts on the development in later steps (Cordeiro et al., 2004; Ponte, 1999). Thus, the composition of the culture medium and environmental factors can result in an intensification of morphogenetic responses as well as a larger number of responsive explants (George, 1996).

The requirement of light during the *in vitro* germination process indicates that most of the evaluated species are positive photoblastic. This result was expected because it seems that light-requiring seeds (which do not germinate under low light conditions) are common of tropical woody species (Chen et al., 2013).

The results support the adoption of tissue culture techniques for the propagation of forest tree species. This biotechnological alternative is associated with the potential of this technique to provide conditions that allow the spread of a wide range of tree species to support reforestation initiatives and forest enrichment. These promising results indicate that in general, the success of micropropagation of woody tree species is associated with the interaction between the type of explant, successful disinfection stage, the adequacy of the conditions of cultivation, the recalcitrance of plant species and, especially, the level of endophytic seed contamination by fungi and bacteria.

Despite this complexity of factors, the results show that more than 80% of the tested species responded under *in vitro* conditions to the propagation method. It should be highlighted that that success in establishing a production routine of woody tree seedlings will not only contribute to the reintroduction of endangered species but will also establish new matrices which in the future may be used for the propagation *in vitro* or *in vivo*. According to George (1996), *in vitro* germination of endangered species helps to ensure genetic variability and may also serve as a source of primary explants for micropropagation.

Conclusion

A single establishment methodology for seed disinfestation (Disinfestation in 70% alcohol and 2.5% sodium hypochlorite) and *in vitro* germination of different tree species (MS or Y3 medium without phytoregulator) can facilitate the simultaneous mass propagation of various tree species to produce seedlings for reforesting regeneration.

References

BERJAK, P.; PAMMENTER, N. W. 2000. What ultrastructure has told us about recalcitrant seeds. Revista Brasileira de Fisiologia Vegetal, v.12 (Edição Especial), p.22-55.

BUTT, M.; USMAN, M.; FATIMA, B. 2013. Enhanced Seed Germination and Callogenesis under long days using Leaf Disc as Explant in *Guava cultivars*. Biologia (Pakistan), v.59, n.2, p.293-298.

CHEN, H.; CAO, M.; BASKIN, J. M.; BASKIN, C. C. 2013. Temperature regulates positively photoblastic seed germination in four *Ficus* (Moraceae) tree species from contrasting habitats in a seasonal tropical rainforest. American Journal of Botany, v.100, n.8, p.1683-1687.

CORDEIRO, I. M. C. C.; LAMEIRA, O. A.; OHASHI, S. T.; ROSAL, L. F. 2004. Efeito de BAP sobre a proliferação de brotos *in vitro* de *Schizolobium amazonicum* Huber ex Ducke (paricá). Cerne, v.10, n.1, p.118-124.

COUTO, J. M. F.; OTONI, W. C.; PINHEIRO, A. L.; FONSECA, E. P. 2004. Desinfestação e germinação *in vitro* de sementes de mogno (*Swietenia macrophylla* King). Revista Árvore, v.28, n.5, p.633-642.

DAFFALLA, H. M.; ELSHEIKH, A. M.; ALI, H. A.; KHALFALA, M. M. 2016. *In vitro* seed germination and node culture of the nutraceutical plant *Grewia tenax*. Environmental and Experimental Biology, v.14, p.75-81.

EEUWENS, C. J. 1976. Mineral requirements for growth and callus initiation of tissue explants excised from mature coconut palms (*Cocos nucifera*) and cultured *in vitro*. Physiologia Plantarum, v.36, p.23-28.

EMMANUEL, E.; KECK, G.; BLANCHARD, J. M.; VERMANDE, P.; PERRODIN, Y. 2004. Toxicological effects of disinfections using sodium hypochlorite on aquatic organisms and its contribution to AOX formation in hospital wastewater. Environment International, v.30, n.7, p.891-900.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. Available in: http://www.agencia.cnptia.embrapa.br/gestor/territorio_mata_sul_pernambucana/arvore/CONT000gx7tnuby02wx7ha0myh2lozpyw5rv.html.

FAO 1993. *Ex situ* storage of seeds, pollen and *in vitro* cultures of perennial woody plant species. FAO Forestry Paper 113. Rome, Italy.

FAY, M. F. 1992. Conservation of rare and endangered plants using *in vitro* methods. *In vitro* Cellular and Developmental Biology, v.28, p.1-4.

FERMINO JR., P. C. P.; PEREIRA, J. E. S.; NAGAO, E. O.; PEDROTTI, E. L.; GUEDES, R. S. 2007. Calogênese e organogênese *in vitro* a partir de segmentos nodais de cerejeira (*Amburana acreana* (Ducke) A.C. Smith) da Amazônia Ocidental: Estabelecimento e regeneração de brotos. Revista Brasileira de Horticultura Ornamental, v.13, p.811-815.

FRANÇA, R. B.; SANTOS, D. S. B.; CABRAL, B. L. R.; MORAES, M.; MOTA, M. G. C.; VIEIRA, I. M. S. 1997. Indução e crescimento de plântulas de pau-rosa (*Aniba roseadora* Ducke) *in vitro*. Anais 2º Reunião dos Botânicos da Amazônia, Salinópolis, PA, Brasil.

GEORGE, E. F. 1996. Plant propagation by tissue culture. England: Exegetics Limited, Second Edition.

GRATTAPAGLIA, D.; MACHADO, M. A. Micropropagação. 1998. In: TORRES, A. C.; CALDAS, L. S.; BUSO, J. A. (org.). Cultura de tecidos e transformação genética de plantas. Brasília: Embrapa-SPI/Embrapa-CNPH, v.1, p.43-76.

LEMOS, O. F; LOPES, S. da C.; MENEZES, I. C.; LAMEIRA, O. A.; OLIVEIRA, M. do S. P. O. 1998. Produção de plântulas para micropropagação do mogno (*Swietenia macrophylla* King). Anais 44 Congresso Nacional de Genética, Águas de Lindóia, SP, Brasil. p.216.

LEN, S. V.; HUNG, Y. C.; CHUNG, D.; ANDERSON, J. L.; ERICKSON, M. C.; MORITA, K. 2002. Effects of Storage Conditions and pH on Chlorine Loss on Electrolyzed Oxidizing (EO). Water. Journal of Agriculture and Food Chemistry, v.50, n.1, p.209-212.

LOPES, S. C. Micropropagação de mogno (*Swietenia macrophylla* King). 2000. Dissertação de Mestrado, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brasil. 53p.

MAIA, G. N. 2006. Tecnologias para a produção agrícola sustentável no semi-árido. In: KÜSTER, A.; MARTÍ, J. F.; MELCHERS, I. (org.). Tecnologias Apropriadas para Terras Secas: Manejo Sustentável de Recursos Naturais em Regiões Semi-áridas no Nordeste do Brasil. Fundação Konrad Adenauer e GTZ. Fortaleza, CE, Brasil. pp.169-172.

MARTINS-CORDER, M. P.; BORGES JUNIOR, N. 1999. Desinfestação e quebra de dormência de sementes de *Acacia mearnsii* de Wild. Ciência Florestal, v.9, n.2, p.1-7.

MURASHIGE, T.; SKOOG, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, v.15, p.473-497.

NASCIMENTO, P. K. V. do; FRANCO, E. T. H.; FRASSETTO, E. G. 2007. Desinfestação e Germinação *in vitro* de Sementes de *Parapiptadenia rigida* Bentham (Brenam). Revista Brasileira de Biociência, v.5, p.141-143.

NUNES, E. da C.; CASTILHO, C. V. de; MORENO, F. N.; VIANA, A. M. 2002. *In vitro* culture of *Cedrela fissilis* Vellozo (Meliaceae). Plant Cell Tissue and Organ Culture, v.70, n.1, p.259-268.

OLIVEIRA, L. S.; DIAS, P. C.; BRONDANI, G. E. 2013. Micropropagação de espécies florestais brasileiras. Pesquisa Florestal Brasileira, v.33, n.76, p.445-460.

PARIHAR, N.; KUMAR, S. 2015. *In Vitro* Seed Germination and Clonal Propagation through Epicotyls Explants of *Aegle marmelos*. International Journal of Research Studies in Biosciences, v.3, n.1, p.67-70.

PASQUAL, M.; CHAGAS, E. A.; SOARES, J. D. R.; RODRIGUES, F. A. 2012. Tissue culture techniques for native Amazonian fruit trees. In: LEVA, A.; RINALDI, L. M. R. Recent Advances in Plant *in vitro* Culture. pp. 151-164.

PÊGO, R. G.; PAIVA, P. D. O.; PAIVA, R. 2013. Micropropagation of *Syngonanthus elegantulus*. Ciência e Agrotecnologia, v.37, n.1, p.32-39.

PONTE, E. M. D. 1999. Micropropagação de *Eucalyptus globulus* sp. Globulus Labill. Dissertação de Mestrado, Universidade Federal de Pelotas. Pelotas, Rio Grande do Sul, Brasil. 47p.

RAM, B.; RATHORE, T. S.; REDDY, G. R. S. 2012. *In vitro* propagation of *Melia dubia* Cav. from seedling explants. Biotechnology Bioinformatics Bioengineering, v.2, n.1, p.610-616.

SANTOS, J. A. 2008. Sistema agroecológico de produção e conservação de forragens na agricultura familiar - a experiência do sertão do pajeú – Pernambuco. Monografia, Universidade Federal de Lavras. Lavras, Minas Gerais, Brasil. 44p.

SARASAN, V. Importance of *in vitro* technology to future conservation programmes worldwide. 2010. Kew Bulletin, v.65, p.549-554.

SILVA, L. C.; PAIVA, R.; VARGAS, P.; SILVA, D. P. C.; BARBOSA, S.; HERRERA, R. C. 2015. Decontaminant solution on *in vitro* growth of *Byrsonima* intermedia seedlings. Ciência Rural, v.45, n.4, p.674-679.

SOUSA, P. B. L.; SANTANA, J. R. F.; CREPALDI, I. C.; LIMA, A. R. 1999. Germination *in vitro* of seeds of a threatened arboreal specie in the municipal district of Abaíra (BA). Sitientibus, n.20, p.89-99.

TASHEVA, K.; KOSTURKOVA, G. 2012. Bulgarian golden root *in vitro* cultures for micropropagation and reintroduction. Central European Journal of Biology, v.5, n.6, p.853-863.

THEILADE, I.; PETRI, L. 2003. Conservation of tropical trees *ex situ* through storage and use. Guidelines and Technical Notes No. 65. Danida Forest Seed Centre, Humlebaek, Dinamarca.

VALVERDE-CERDAS, L.; DUFOUR, M.; VILLALOBOS, V. 1998. *In vitro* organogenesis in *Albizia guachapele*, *Cedrella odorata* and *Swietenia macrophylla* (Fabaceae, Meliaceae). Revista de Biologia Tropical, v.46, n.2, p.225-228.

VENGADESAN, G.; PIJUT, P. M. 2009. *In vitro* propagation of northern red oak (*Quercus rubra* L.). In Vitro Cellular & Developmental Biology-Plant, v.45, p.474-482.

YANG, G.; SHEN, X.; JACKSON, R.; Lu, Z. 2013. Factors affecting *in vitro* seed germination and shoot proliferation of galax [*Galax urceolata* (Poir.) Brummitt]. Australian Journal of Crop Science, v.7, n.11, p.1766-1771.