In vitro germination and cryopreservation of *Zinnia elegans* seeds [1]

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ABSTRACT

Zinnia elegans Jacquim is a species of big cutting and ornamental potential, but its seeds have low germination percentage. This study aimed to establish a protocol for *in vitro* germination and water content for the cryopreservation of seeds. The length, width, thickness, and weight of one thousand seeds were determined. MS and WPM culture mediums, as well as the concentrations of gibberellic acid (GA_3) were tested regarding *in vitro* culture. For cryopreservation, the seeds were subjected to drying on silica gel or laminar flow for different times. Then, the seeds were stored in liquid nitrogen (-196° C) for 24 hours. After this period, the seeds were thawed and inoculated into culture medium. The biometric results of seeds showed 8.6 mm average length, 4 mm width, and 0.9 mm thickness. The weight of one thousand seeds was 851 mg, characterizing them as small, lightweight, and easy to disperse. The use of MS medium with no addition of GA_3 enhanced germination (67%). The initial moisture content of *Z. elegans* seeds was 9%. Seeds subjected up to 2 hours of drying in both treatments obtained 23% germination in silica gel and 19% in laminar flow. *Z. elegans* seeds may be desiccated by 4% moisture content and cryopreserved with no loss of germination potential.

Keywords: *In vitro* conservation, ornamental plant, tissue culture.

RESUMO

Germinação *in vitro* e criopreservação de sementes de *Zinnia elegans*

Zinnia elegans Jacquim é uma espécie de grande potencial para flor de corte e ornamentação, porém suas sementes apresentam baixa porcentagem de germinação. Objetivou-se estabelecer um protocolo para germinação *in vitro* e o teor de água para a criopreservação das sementes. O comprimento, largura e espessura das sementes e o peso de mil sementes foi determinado. Quanto ao cultivo *in vitro* testou-se os meios de cultura MS e WPM e cinco concentrações de ácido giberélico (GA_3). Para a criopreservação, as sementes foram submetidas à secagem em sílica gel ou fluxo laminar por diferentes tempos. Em seguida, as sementes foram armazenadas em nitrogênio líquido (-196º C) por 24 horas, após esse período, as sementes foram descongeladas e inoculadas em meio de cultura. Os resultados de biometria das sementes apresentaram comprimento médio (mm) de 8,6; largura de 4,0 e espessura de 0,9. O peso de mil sementes foi de 851 mg, caracterizando-as como pequenas, leves e de fácil dispersão. A utilização do meio de cultura MS sem adição de GA_3 favoreceu a germinação *in vitro* (67%). O teor de água inicial das sementes de *Z. elegans* foi de 9%. As sementes submetidas até 2 horas de secagem em ambos os tratamentos obtiveram germinação de 23% na sílica gel e 19% no fluxo laminar. Sementes de *Z. elegans* podem ser dessecadas até graus de umidade em torno de 4%, e criopreservadas sem perda do poder germinativo.

Palavras-chave: Conservação *in vitro*, planta ornamental, cultura de tecidos.

1. INTRODUCTION

The production of flowers and ornamental plants has been increasing in Brazil in recent years due to the enlargement of consumption in developed countries, mostly because of the expansion in Brazilian domestic market (MELO et al., 2014). According to the estimates of *Instituto Brasileiro de Floricultura* (IBRAFLOR, 2013), the growth of the floriculture sector was from 10 to 15% in the last years, revolving around 4.4 billion Brazilian reals a year. Therefore, it requires more and more investment in technology and farming techniques to maximize the ornamental flower production seeking phytosanitary quality and long-term investment in storage of species with high annual demand, thus, increasing the production of seedlings, leaves, and flowers which satisfactorily meet the market requirements.

Among ornamental plants with cutting potential, *Z. elegans* stands out since it presents a wide variety of colors, flowers, and petal format (TORRES, 1963) as well as the possibility to be grown throughout the year (STIMART et al., 1987). Guimarães et al. (1998) indicate in their studies that it is difficult to obtain *Z. elegans* seeds with high vigor and uniform physiological age because they have a long flowering period, specially taking into account that inflorescences of different age and size are harvested

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together. Owing to this, *Z. elegans* seeds usually have low germination percentage notedly when it is directly sowed on field. In such conditions, the highest germination and vigor were achieved after 50 days following anthesis with 45% germination (GUIMARÃES et al., 1998). Thus, the use of *in vitro* cultivation techniques may improve the germination rate in a shorter period of time, using less space to obtain more uniform seedlings and better phytosanitary quality (CARVALHO et al., 2012).

Studies on the biometric aspects, physiological potential, and sanitary status of a seed sample along with the modifications that may occur in germination and water content during the development are of great importance both for the propagation and long-term storage (GUIMARÃES et al., 1998; OLIVEIRA et al., 2012). As a strategy for long-term storage, the cryopreservation technique stands out. It consists of keeping biological material alive for an indefinite period of time in an ultra-low temperature (from -150º C to -196º C) (ENGELMANN, 2011).

The cryopreservation provides advantages such as totally inhibiting chemical reactions which may hazard cells (MAZUR, 1984) as well as the action of internal and external agents that may affect the integrity of seeds, maintaining the genetic integrity of conserved material, too (STANWOOD, 1985). It is an alternative to preserve species which are kept in field collections, botanical gardens, nature reserves or in *in vitro* conservation systems with plants in controlled growth regimen ((SARASAN et al., 2006; PÉREZ-MOLPHE-BALCH et al., 2012). In face of the above mentioned, the aim was to establish a protocol for *in vitro* germination and the determination of the water content limit for the cryopreservation of *Z. elegans* seeds.

2. MATERIAL AND METHODS

Seeds were collected between November and December 2012 in *Floresta Nacional de Ritapólis* (FLONA) in Ritapólis, MG, Brazil, during natural maturation and dispersal of seeds. After collecting, the weight of one thousand seeds was determined according to *Regras para Análise de Sementes* (BRASIL, 2009) using a 0.05 mm digital caliper to accurately determine length, width, and thickness of 100 seeds. The means and standard deviation were calculated for each parameter.

Seeds were taken to the laminar flow chamber, immersed in 70% alcohol for 1 minute and, afterwards, in sodium hypochlorite solution (NaOCl) with 1% active chlorine for 10 minutes. Subsequently, seeds were rinsed three times with distilled water, and inoculated in different culture medium. MS (MURASHIGE and SKOOG, 1962) medium and *Woody Plant Medium* (WPM) (LLOYD and MCCOWN, 1981) were used. It was also tested five concentrations of giberellic acid (GA_3) (0.0; 5.57; 11.54; 17.31; 23.08 µM) supplied to MS medium, added with 30 g L^{-1} sucrose and 7 g L^{-1} agar. The pH of the medium was adjusted to 5.8 before autoclaving at 121º C during 20 minutes. After inoculation, seeds were kept in growth chamber at 36 µmol m^{-2} s^{-1} photons irradiance, 25° C ± 2° C temperature, and 16-hour photoperiod. Each treatment consisted of 25 seeds and the evaluation of germination was carried out in 2-day intervals for 45 days recording the percentage of seeds with 2 mm rootlets in each treatment and the Germination Speed Index (GSI) calculated according to Maguire (1962).

Seeds had initial moisture content determined by the method of fast drying in an oven at 105º C ± 2º C temperature for 24 hours (BRASIL, 2009), using five subsamples with 10 seeds each one. Seeds were subjected to drying in silica gel (100g) and laminar flow for different periods of time (0, 1, 2, and 3 hours). Twenty-five seeds were used for each treatment. Ten seeds out of 25 were placed in cryotubes and immersed in liquid nitrogen (LN) (-196º C) for 24 hours, ten seeds were laid on MS medium (germination control), and five were used to determine water content. After 24 hours in LN, seeds passed through the heating process in a water bath at 38º C ± 2º C temperature for four minutes. Then, inoculated in MS medium and kept in growth chamber under 36 µmol m^{-2} s^{-1}irradiance, 16-hour photoperiod, and 25° C ± 2° C temperature. The evaluation of the germination was carried out along 45 days recording the percentage of seeds with 2 mm rootlet in each treatment.

A completely randomized design was used. Analysis of variance was performed with SAS® statistical software (SAS, 1993), comparing the frequencies by Fischer's exact test at 5% probability.

3. RESULTS

Regarding seeds biometry, they had 8,6 ± 0,910 average length (mm) and standard deviation, 4,0 ± 0,475 width, 0,9 ± 0,193 thickness. It was observed that the standard deviation values were relatively low, indicating the high homogeneity of the sample. Seeds had dry integument with visible and light hila, oblong geometric shape and brown color (Figure 1). The weight of one thousand seeds was 0,851g ± 0,048, characterizing them as small, lightweight, and ease to disperse as described in *Regras para Análise de Sementes* (BRASIL, 2009).

Results showed that MS medium presented higher germination percentage (66.67%) for *Z. elegans* seeds (Figure 2).

Z. elegans seeds germinated in MS medium from the 13th day on. Plants presented normal aspects and exhibited shoots and roots well-developed at 40 days (Figure 3).

The germination percentage and the germination speed index (GSI) were higher when seeds were inoculated in MS medium with no GA_3 addition, exhibiting 60% germination percentage and 0.03 GSI (Table 1).

Figure 1. Overview of *Z. elegans* seeds showing its shape and size. Bar = 0.5cm.
Figura 1. *Aspecto geral da semente de Z. elegans evidenciando sua forma e tamanho. Barra= 0,5cm;*

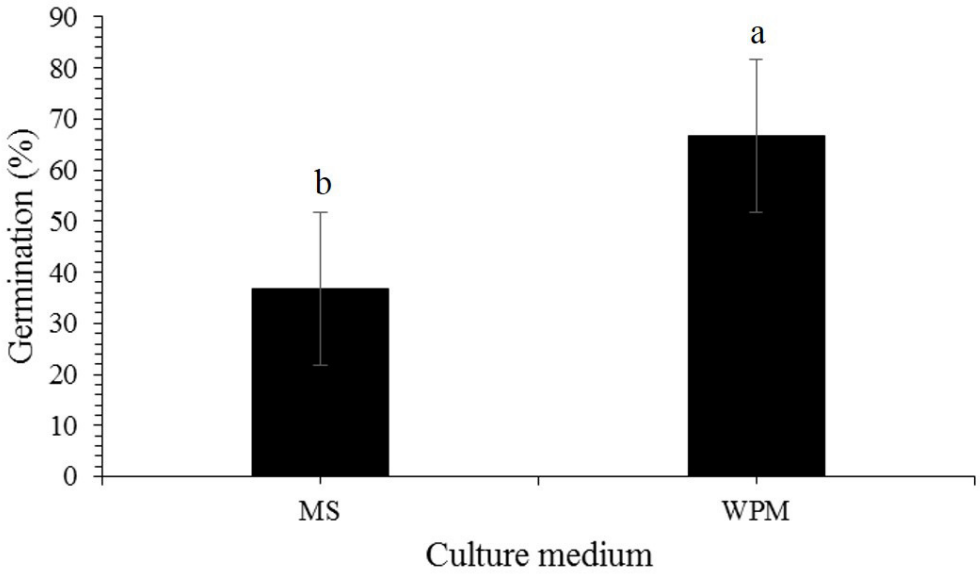

Figure 2. Germination percentage of *Z. elegans* seeds in different culture medium (MS and WPM). Bars followed by different letters show a significant range between treatments and bars in the graphs represent the average standard deviation according to Fischer's exact test (P≤0.05). (n = 30).
Figura 2. *Porcentagem de germinação de sementes de Z. elegans em diferentes meios de cultura (MS e WPM). Barras seguidas de letras diferentes mostram uma variação significativa entre os tratamentos e as barras nos gráficos representam o erro padrão da média de acordo com o teste exato de Fisher (P ≤ 0,05). (n = 30).*

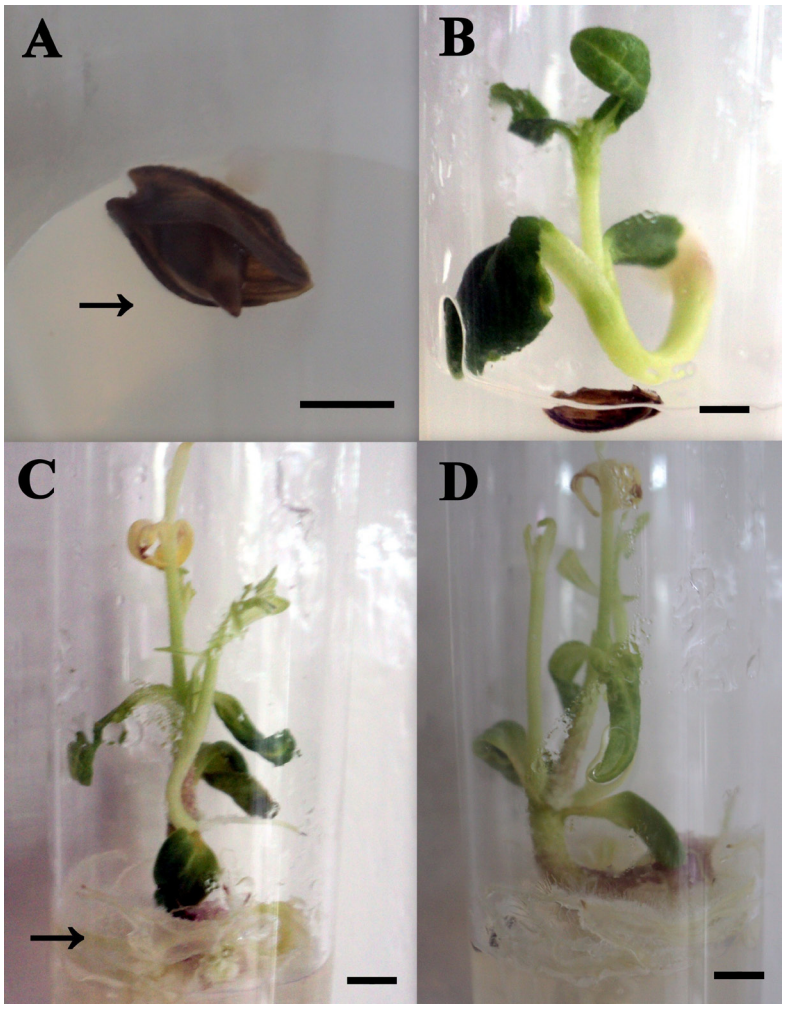

Figure 3. *Z. elegans* seeds inoculated onto MS culture medium at 13 days after inoculation, observing the protruded rootlet (arrow) (A) and at 21 days with the development of primary root (B). Development of secondary roots (arrow) (C) and formed plant at 40 days after *in vitro* germination (D).

Figura 3. *Sementes de Z. elegans inoculadas em meio de cultura MS aos 13 dias após a inoculação observando a radícula protrundida (seta) (A) e aos 21 dias com o desenvolvimento de raiz primária (B). Desenvolvimento de raízes secundárias (seta) (C) e a planta formada aos 40 dias após a germinação in vitro (D).*

Table 1. Germination percentage of *Z. elegans* seeds inoculated on MS medium supplied with different concentrations of GA_3.

Tabela 1. *Porcentagem de germinação de sementes de Z. elegans inoculadas em meio MS suplementado com diferentes concentrações de* GA_3.

Treatment (µM)	Germination (%)	GSI
0,0	60,00 a	0,03 a
5,57	30,00 b	0,03 a
11,54	20,00 c	0,02 b
17,31	10,00 d	0,02 b
23,08	20,00 c	0,02 b

* Averages followed by the same lower case in column do not differ between them by Fischer's exact test at 5% probability.

** Médias seguidas pela mesma letra minúscula na coluna não diferem estatisticamente pelo teste de exato de Ficher com 5% de probabilidade.*

The initial water content of *Z. elegans* seeds was 9%. After 3 hours dehydration in silica gel and laminar flow, the water content was 4.1% and 3.3%, respectively. There was a low germination percentage for seeds subjected up to 2-hour drying and cryopreservation, with 22.75% silica and 18.18% laminar flow values when compared to controls (Table 2). Thus, *Z. elegans* seeds may be dissected in moisture levels by 4% and subsequently cryopreserved with no loss of the germination potential.

Table 2. Percentage of moisture content and germination of *Z. elegans* seeds subjected to drying in silica gel and laminar flow for different times, immersed in liquid nitrogen (+LN) or without passing through the liquid nitrogen (-LN).
Tabela 2. *Porcentagem do teor de umidade e germinação das sementes de Z. elegans submetidas a secagem em sílica e no fluxo laminar por diferentes tempos, imersas em nitrogênio líquido (+NL) ou sem passar pelo nitrogênio líquido (-NL).*

Time	Moisture content (%)		Germination (%)			
	Silica	Flow	Silica (-LN)	Flow(-LN)	Silica (+LN)	Flow (+LN)
Control	8,791	9,015	33,33a C	75,00a A	31,82a C	45,45a B
1 hour	7,142	6,518	33,33a C	55,00a A	31,82a C	36,36a B
2 hours	6,043	4,354	33,33a A	33,33b A	22,73b B	18,18b C
3 hours	4,129	3,328	00,00b B	00,00c B	13,64c A	00,00c B

***** Averages followed by the same lower case in column do not differ between them by Fischer's exact test at 5% probability.
** Médias seguidas pela mesma letra minúscula na coluna não diferem estatisticamente pelo teste de exato de Ficher com 5% de probabilidade.*

4. DISCUSSION

The light weight observed in *Z. elegans* seeds makes it easy to disperse, since it can be considered anemocoric. However, as the flowering and dispersion period is longstanding, there are some adjusts in the number of seeds produced by the mother plant to maintain a relatively constant supply of assimilated (MARCOS FILHO, 2005).

The higher percentage using MS medium may be explained due to the existence of a greater macronutrient availability in the formula, with high concentrations of total nitrogen, calcium, manganese, and zinc, besides micronutrients, vitamins, and amino acids required for the germination process (PINHEIRO et al., 2001; MOREIRA et al., 2012). The culture medium WPM has lower concentrations of total nitrogen (ROCHA et al., 2007) and only 45% of the total ionic strength of MS medium (NUNES et al., 2002; OLIVEIRA et al., 2011). Thus, it is important to know the nutritional requirements which affect the germination of each species seeds to be successful at establishing *in vitro* plants.

The presence of GA_3 had no beneficial effects on the germination of *Z. elegans* seeds, which means that the concentrations of gibberellins did not promote positive responses. These results corroborate those found by Carvalho et al. (2012) who worked with *Passiflora gibertii* seeds cultivated in culture medium with GA_3 and found that the seeds were able to germinate regardless the addition of the growth regulator. Valio (1976) described that the gibberellin when applied on the culture medium where seeds have already high concentrations of endogenous gibberellin may delay root protusion, and the decrease of germination might be related to the inhibition of hydrolytic enzymes by products of reactions catalyzed by enzymes induced by endogenous gibberellin.

For the long-term storage of seeds, the survival after thawing depends on the quality, physiological status, and water content of seeds (HUEHNE and BHINIJA, 2012). The water content is a meaningful factor for the successful cryopreservation. Thus, it is essential to prevent the formation of ice crystals in the intracellular environment, protecting the embryo against damages which might be lethal (HIRANO et al., 2005). The temperature between -15º C and -60º C is considered to be critical, since this is the range in which there is nucleation and formation of ice crystals from the free water in cells. Additionally, the explant is subjected twice to these temperature ranges, the first one during cooling, and the second one during reheating (MAZUR, 1984).

Thereby, seed dissection has advantages like: it is a simple, non-toxic, and low cost method. However, it is important to set the dehydration threshold for each species because the excessive dehydration may cause losses in germination potential as a consequence of damages related to the metabolism of the embryo (PAMMENTER et al., 1998; WALTERS et al., 2001; BERJAK et al., 2012).

In the present study, *Z. elegans* seeds subjected to desiccation for up to 2 hours in silica gel and laminar flow presented 6% and 4.3% water content, respectively, and germinated after cryopreservation. These are the water ranges in which cryopreservation is considered to be a proper method to store the seeds of this species (CAVALCANTI-MATA, 2001; ENGELMANN, 2011).

For many terrestrial and epiphytes species, cryopreservation depends on the water content being reduced to a critical point (PRITCHARD, 1984; PRITCHARD et al., 1999; NIKISHINA et al., 2001;). In *Dendrobium candidum* (Orchidaceae) seeds, for instance, a high survival rate (around 95%) was obtained when the water content of seeds decreased to 8% and 9% before cryopreservation (WANG et al., 1998). A high survival rate was also obtained in *Bletilla formosana* seeds when the water content decreased from 24.8% to 1.9% (HU et al., 2013).

5. CONCLUSIONS

The *in vitro* cultivation of *Z. elegans* on MS medium with no addition of growth regulator GA_3 favors seed germination with a 67% germination percentage. *Z. elegans* seeds can be cryopreserved with a water content threshold between 6% and 4%.

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REFERENCES

BERJAK, P.; PAMMENTER, N.W.; WESLEY-SMITH, J. The effects of various parameters during processing for cryopreservation on the ultrastructure and viability of recalcitrant zygotic embryos of *Amaryllis belladonna*. **Protoplasma**, Wien, v.249, n.1, p.155-169, 2012. DOI: 10.1007/s00709-011-0274-5

BRASIL. Ministério da Agricultura. **Regras para Análise de Sementes**. Divisão de sementes e mudas. Brasília, DF. s.n. 2009. 399p. vol. 2.

CARVALHO, M.A.F.; PAIVA, R.; VARGAS, D.P.; PORTO, J.M.P.; HERRERA, R.C.; STEIN, V.C. Germinação *in vitro* de *Passiflora gibertii* N. E. Brown com escarificação mecânica e ácido giberélico. **Semina: Ciências agrárias**, Londrina, v.33, n.3, p.1027-1032, 2012. DOI: http://dx.doi.org/10.5433/1679-0359.2012v33n3p1027

CAVALCANTI MATA, M.E.R.M. **Crioconservação dos recursos fitogenéticos de espécies florestais, medicinais e de interesse econômico do semi-árido do Nordeste do Brasil.** Campina Grande: UFCG, 2001. 68p. vol.1.

ENGELMANN, F. Use of biotechnologies for the conservation of plant biodiversity. ***In vitro* Developmental Biology**, New York, v.47, n.4, p.5-16, 2011. DOI: 10.1007/s11627-010-9327-2.

GUIMARÃES, T.G.; OLIVEIRA, D.A.; MANTOVANI-ALVARENGA, E.; GROSSI, J.A.S. Maturação fisiológica de sementes de zínia (*Zinnia elegans* Jacq.). **Revista Brasileira de Sementes**, v.20, n.1, p.7-11, 1998. DOI: http://dx.doi.org/10.1590/S0101-31222008000300022

HIRANO, T.; GODO, T.; MII, M.; ISHIKAWA, K. Cryopreservation of immature seeds of *Bletilla striata* by vitrification. **Plant Cell Reports**, New York, v.23, n.8, p.534-539, 2005. DOI: 10.1007/s00299-004-0893-9

HU, W.H.; YANG, Y.H.; LIAW, S.I.; CHANG, C. Cryopreservation the seeds of a Taiwanese terrestrial orchid, *Bletilla formosana* (Hayata) Schltr. by vitrification. **Botanical Studies**, New York, v.54, n.1, p.33, 2013. DOI: 10.1186/1999-3110-54-33

HUEHNE, P.S.; BHINIJA, K. Application of cryoprotectants to improve low temperature storage survival of orchid seeds. **Scientia Horticulturae**, Amsterdam, v.135, n.1, p.86-193, 2012. DOI: 10.1016/j.scienta.2011.11.026

IBRAFLOR - Instituto Brasileiro de Floricultura. **Uma visão do Mercado de flores,** 2013. Disponível em: <hppt://www.ibraflor.com/publicacoes/vw.php?cod=21>. Acesso em: 15 de Agosto de 2014.

LLOYD, G.; MC COWN, B. Use of Microculture for production and improvement of *Rhododendro* spp. **Hortscience**, Alexandria, v.15, p.412-416, 1980. DOI: http://dx.doi.org/10.1590/S0103-84782010005000084

MAGUIRE, J.B. Speed of germination-aid in selection and evaluation for seedling emergence vigor. **Crop Science**, Madison, v.2, n.2, p.176-177, 1962. DOI: 10.2135/cropsci1962.0011183X000200020033x

MARCOS FILHO, J. **Fisiologia de sementes de plantas cultivadas**. Piracicaba: Fealq. 2005. 495p., vol. 1.

MAZUR, P. Freezing of living cells: mechanisms and implications**. American Journal of Physiology-Cell Physiology**, Bethesda, v.247, n.3, p.125-142, 1984.

MELO, L.F.; GOMES, R.L.F.; DA SILVA, V.B.; MONTEIRO, E.R.; LOPES, Â.C.A.; PERON, A.P. Potencial ornamental de acessos de pimenta. **Ciência Rural**, Santa Maria, v.44, n.11, p. 2010-2015, 2014. DOI: http://dx.doi.org/10.1590/0103-8478cr20131306

MOREIRA, R.A.; RODRIGUES, F.A.; MONFORT, L.E.F.; PIRES, M.F.; PASQUAL, M. Diferentes meios de cultura no crescimento *in vitro* de sorvetão. **Brazilian Journal of Agricultural Sciences**, Recife, v.7, n.3, p.409-413, 2012. DOI: 10.5039/agraria.v7i3a1447

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bioassays with Tobacco tissue cultures. **Physiologia Plantarum,** Copenhagen, v.15, n.3, p.473-497, 1962. DOI: 10.1111/j.1399-3054.1962.tb08052.x

NIKISHINA, T.V.; POPOV, A.S.; KOLOMEITSEVA, G.L.; GOLOVKIN, B.N. Effect of cryopreservation on seed germination of rare tropical orchids. **Russian Journal of Plant Physiology,** New York, v.48, n.6, p.810-815, 2001. DOI: 10.1023/A:1012520927743

NUNES, E.C.; DE CASTILHO, C.V.; MORENO, F.N.; VIANA, A.M. *In vitro* culture of *Cedrela fissilis* Vellozo (Meliaceae). **Plant Cell, Tissue And Organ Culture**, Dordrecht, v.70, n.3, p.259-268, 2002. DOI: 10.1023/A:1016509415222

OLIVEIRA, M.L.D.; XAVIER, A.; FILHO, R.M.P.; OTONI, W.C.; TEIXEIRA, J.B. Efeitos do meio de cultura e da relação BAP/ANA na multiplicação *in vitro* de clones de *Eucalyptus grandis* x *E. urophylla* em biorreator de imersão temporária. **Revista Árvore**, Viçosa, v.35, n.6, p.1207-1217, 2011. DOI: http://dx.doi.org/10.1590/S0100-67622011000700007

PAMMENTER, N.W.; GREGGAINS, V.; KIOKO, J.I.; WESLEY-SMITH, J.; BERJAK, P.; FINCH-SAVAGE, W.E. Effects of differential drying rates on viability retention of *Ekebergia capensis*. **Seed Science Research**, Cambridge, v.8, n.4, p.463-471, 1998. DOI: http://dx.doi.org/10.1017/S0960258500004438

PÉREZ-MOLPHE-BALCH, E.; PÉREZ-REYES, M.E.; ROSA-CARRILLO, M.L.L. *In vitro* conservation of *Turbinicarpus* (Cactaceae) under slow growth conditions. **Haseltonia,** Washington, v.17, n.1, p.51-57, 2012. DOI: http://dx.doi.org/10.2985/1070-0048-17.1.6

PINHEIRO, C.S.R.; MEDEIROS, D.D.; MACEDO, C.D.; ALLOUFA, M.A.I. Germinação i*n vitro* de mangabeira (*Hancornia Speciosa* Gomez) em diferentes meios de cultura. **Revista Brasileira de Fruticultura**, Jaboticabal, v.23, n.2, p.145-151, 2001. DOI: http://dx.doi.org/10.1590/S0100-29452001000200043

PRITCHARD, H.W. Liquid nitrogen preservation of terrestrial and epiphytic orchid seed. **CryoLetters,** London, v.5, n.1, p.295–300, 1984.

PRITCHARD, H.W.; POYNTER, A.L.C.; SEATON, P.T. Interspecific variation in orchid seed longevity in relation to ultra-dry storage and cryopreservation. **Scientific Journal of the American Orchid Society**, v.14, n.2, p.92-101, 1999.

ROCHA, S.C.D.; QUORIM, M.; RIBAS, L.L.F.; KOEHLER, H.S. Micropropagation of *Cabralea canjerana*. **Revista Árvore**, Viçosa, v.31, n.1, p.43-50, 2007. DOI: http://dx.doi.org/10.1590/S0100-67622007000100006

SARASAN, V.; CRIPPS, R.; RAMSAY, M.M.; ATHERTON, C.; MCMICHEN, M.; PRENDERGAST, G.; ROWNTREE, J.K. Conservation *in vitro* of threatened plants-progress in the past decade. **In Vitro Cellular & Developmental Biology-Plant**, New York, v.42, n.3, p.206-214, 2006. DOI: 10.1079/IVP2006769

SAS Institute. **SAS User's Guide: statistics.** Cary, USA: SAS Inst., 1993.

STANWOOD, P.C. **Cryopreservation of seed germplasm for genetic conservation.** In: KARTHA, K.K. (ed.), Cryopreservation of plant cells and organs. CRC, Boca Raton, Florida, 1985. p. 199-226.

STIMART, D.P.; BOYLE, T.H.; TERRY-LEWANDOWSKI, V.M. Genetic and physiological studies of *Zinnia elegans*, *Z. angustifolia* and their interspecific hybrids. **Horticulture Science**, Alexandria. v.22, n.526, p.690-69, 1987.

TORRES, A.M. Taxonomy of *Zinnia.* **Brittonia,** New York, v.15, p.1-25, 1963. DOI: 10.2307/2805035

VALIO, I.F.M. Germination of coffee seeds (*Coffea Arabica* L.) cv. Mundo Novo. **Journal of Experimental Botany**, Oxford, v.27, n.5, p.983-991, 1976. DOI: 10.1093/jxb/27.5.983

WALTERS, C.; PAMMENTER, N.W.; BERJAK, P.; CRANE, J. Desiccation damage, accelerated aging and respiration in desiccation tolerant and sensitive seeds. **Seed Science Research**, Cambridge, v.11, n.2, p.135-148, 2001. DOI: http://dx.doi.org/10.1079/SSR200168

WANG, J.H.; GE, J.G.; LIU, F.; BIAN, H.W.; HUANG, C.N. Cryopreservation of seeds and protocorms of *Dendrobium candidum*. **CryoLetters**, London, v.19, n.1, p.123-128, 1998.