Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
www.ufersa.edu.br/caatinga

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)

IN VITRO SEED GERMINATION OF MANDACARU (*Cereus jamacaru* DC.)[1]

MAILSON MONTEIRO DO RÊGO[2], EMMANUELLE RODRIGUES ARAÚJO[3*], ELIZANILDA RAMALHO DO RÊGO[4], JULIANA PEREIRA DE CASTRO[2]

ABSTRACT - Mandacaru (*Cereus jamacaru* DC.) is a native cactus of Caatinga with a big importance for development sustainable and biodiversity conservation of this biome. The goal of this work was to disinfest and promoter seed germination of this specie in vitro. For this different concentration of the sodium hypochlorite solution and sucrose were evaluated. The experimental design was completely randomized in factorial 5 x 5, with five replicates by treatments. Treatments consisted in five sodium hypochlorite concentration (0.0; 0.5; 1.0; 1.5 and 2.0%) and five sucrose concentration (0.0; 2.5; 5.0; 7.5 and 10.0 $g.L^{-1}$). The following variables were analyzed: contamination; seed germination frequency and seedling growth. The analysis of variance shown that there was a significant interaction for seed germination at 5% of probability by F test. The 2.5% sucrose supplemented media was the most efficient treatment. On other hand, it was observed that the concentration of sodium hypochlorite soluction at 0.5% was effective in seed disinfestations. The best treatment was at 1.0% of hypochlorite. Regarding the seedling growth there was no significant differences among treatments.

Keywords: Cactaceae. Tissue culture. Caatinga. Sucrose.

GERMINAÇÃO *IN VITRO* DE SEMENTES DE MANDACARU *(Cereus jamacaru* DC.)

RESUMO - O mandacaru (*Cereus jamacaru* P. DC.) é uma cactaceae nativa da caatinga, possuindo grande importância para a sustentabilidade e conservação da biodiversidade deste bioma. Objetivou-se com este trabalho desinfestar e promover a germinação de sementes de mandacaru, variando os fatores concentração de hipoclorito de sódio e concentração de sacarose. O delineamento experimental foi inteiramente casualizado, em esquema fatorial 5x5, com cinco repetições. Os tratamentos consistiram de cinco concentrações de hipoclorito de sódio (0,0; 0,5; 1,0; 1,5 e 2,0% de cloro ativo) e cinco concentrações de sacarose (0,0; 2,5; 5,0; 7,5 e 10,0%). Foram avaliados o número de contaminações, germinações e tamanho da planta por semente cultivada. Houve interação significativa, apenas para a variável germinação, sendo a concentração de 2,5% de sacarose a mais eficiente dentro das quatro concentrações de cloro ativo. Verificou-se que a concentração de cloro ativo a partir de 0,5% é efetiva na desinfestação das sementes, sendo a concentração de 1,0% o melhor tratamento. Com relação à característica comprimento de plântula não houve diferenças significativas para os dois fatores analisados.

Palavras-chave: Cactaceae. Cultura de tecido. Sacarose.

[*]Autor para correspondência**.**
[1]Recebido para publicação em 13/08/2008; aceito em 25/05/2009.
[2]Depto. de Fitotecnia, CCA/Universidade Federal da Paraíba (UFPB), Caixa Postal 04, 58.397-000, Areia–PB
[3]Departamento de Fitossanidade, Universidade Federal Rural de Pernambuco (UFRPE), rua Dom Manoel de Medeiros, s/n, Dois Irmãos, 52171-900, Recife–PE; manucg@gmail.com
[4]Depto. de Ciências Fundamentais e Sociais, UFPB, Areia–PB

INTRODUCTION

The name Cacti or Cactus has been adopted as the universal term to describe succulent plants. Their use is one of the most important connections between humans and plants in the dryland region of Brazil. It requires low inputs of water and fertilizers to provide food and fodder for sustainable development in arid and semiarid regions. It is being grown worldwide for its delicious sweet fruits, vegetable, nutritive forage, and for several other medicinal as well as industrial uses (ANDRADE et al., 2006).

The xerophytes plant community called Caatinga, found all over an area of approximately 800,000 sq km in northeastern Brazil, lies within the 800 mm isohyets of annual rainfall. The Caatinga is a biome with restricted occurrence to Brazil, and in spite of its socioeconomic importance, it has being submitted for decades to intense and predatory exploration of its natural resources, and it has not been very protected and studied (ANDRADE et al., 2006).

Cereus jamacaru De Candolle, Cactaceae, popularly known as mandacaru, is a common columnar cactus in Brazil, mainly in the Caatinga Semi-Arid Region with a great importance for development sustainable and biodiversity conservation of this biome. It is especially important during the driest months due to its use as cattle food. The stems are used to treat several diseases (CAVALCANTE; RESENDE, 2007).

The species of succulent plants are very susceptible to rots caused by bacteria and fungi. These ones affect the germinative vigor of seeds and can promote the abnormal growing of plantlets in tissue culture. Methods for seed disinfestations in micropropagation of cacti have been developed to overcoming these problems mentioned above and to obtain healthy plants (COUTO et al., 2004).

The success of micropropagation involves several factors, like the composition of the culture medium, culture environment, and genotype. The development of procedures for rapid *in vitro* clonal micropropagation of cactus may have a great commercial value to the agriculture. Tissue culture techniques should minimize the time necessary for the introduction of new cultivars into the commercial market and so increase the availability of plants with improved horticultural characteristics (BRESSAN et al., 1982).

Sucrose is by far the most used carbon source, for several reasons. It is cheap, readily available, relatively stable to autoclaving, and readily assimilated by plants. Other carbohydrates can be also used, such as glucose, maltose and galactose as well as the sugar-alcohols glycerol and sorbitol (FOWLER, 2000). The carbohydrates added to the culture medium supply energy for the metabolism (CALDAS et al., 1998). The addition of a carbon source in any nutrient medium is essential for *in vitro* growth and development of many species, because photosynthesis is insufficient, due to the growth taking place in conditions unsuitable for photosynthesis or without photosynthesis (in darkness).

The goal of this work was to disinfest and to promote seed germination of Mandacaru (*Cereus jamacaru* P. DC.) specie *in vitro*.

MATERIAL AND METHODS

Plant Material

Seeds were obtained from mature fruits from Galant District, Paraíba State, Brazil. Surface sterilization was realized in the Plant Biotechnology Laboratory at Universidade Federal da Paraíba.

Surface Sterilization Treatments

On laminar flowhood using a Becker, the ripe fruits were surface sterilized for 1 min in 70% ethanol solution, followed rinsing with sterile deionized water and dipped in a sodium hypochlorite solution at different concentrations: 0.0; 0.5; 1.0; 1.5 and 2.0% with three drop of Tween 20/ 100 mL for 10 minutes. After this treatment, the seeds were washed by four times in distillated, deionized and autoclaved water and placed on a paper filter to dry off the excess water.

Culture Medium Treatments

Seeds were inoculated in MS medium (MURASHIGE; SKOOG, 1962) with different concentrations of sucrose (0.0; 2.5; 5.0; 7.5 and 10.0 $g.L^{-1}$) plus 0.8% of agar. The pH was adjusted to 5.7 with NaOH (MILANESE, 1997). Ten milliliters of medium were autoclaved for 20 minutes at 120 °C in glass tubes. The tubes were tilted; thus, when the medium cooled down a larger surface was formed, the seeds were sown and kept in the dark for 15 days at 25±2 ºC. After this, the tubes were moved to a room with 16 hours under light e 8h under darkness with a luminosity of 30µmol m^{-2} s. The swollen seeds with green embryos were observed with a stereomicroscope and were considered as germinated when presented plantlets (HAILES; SEATON, 1989).

Data analysis

The data were submitted to the square root transformation like proposed by Bartlett, 1936. The experimental design was completely randomized and the data were analysed in a factorial experiment 5 x 5, with five replicates for treatments. The following variables ere evaluated: contamination, germination seed frequency and seedling growth. The results were submitted to statistical ANOVA analysis and followed by Duncan's multiple-range test ($a<0.05$).

RESULTS AND DISCUSSION

The results of ANOVA revealed presence of interaction between sucrose concentrations and sodium hypochlorite concentrations to the germination variable but not for the other. The factors affect independently the seed contamination and should be analyzed separately. There were no significant differences to the seedling growth (Table 1). The means of all significant differences using comparison of means by Duncan's method are showed in Tables 2 and 3. The germination started

Table 1. Analysis of variance to variables contamination, germination and seedling growth in Mandacaru (*Cereus jamacaru* P. DC).

Source of variation	D.F.	Mean Square		
		Contamination	Germination	Seedling growth
Sucrose (S)	4	161,616.11^{ns}	1,406.658.78*	9,166527.10^{ns}
Sodium Hipoclorite (NaHClO)	4	1,179199.07*	508.791,48^{ns}	91,703.104.06^{ns}
(NaHClO) x S	16	303,778.44^{ns}	344,182.47*	91,707934.26^{ns}
Resíduo	100	281,331.76	167,601.90	91,712383.52
Total	124			

* Significant (α<0.05) by F statistics; ^{ns} nonsignificant.

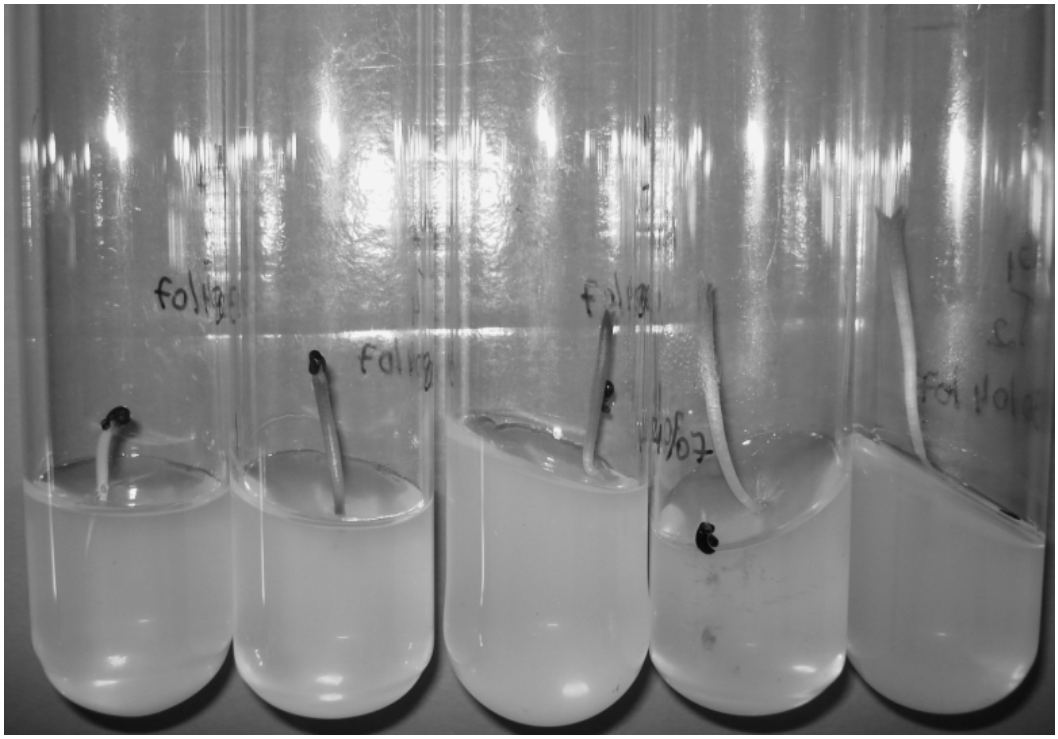

Figure 1. Germination of *Cereus jamacaru* seeds.

The Duncan's test showed that in absence of sodium hypochlorite the seeds germination did not depend on sucrose concentrations. In the 0.5; 1.0 e 1.5 of hypochlorite the best sucrose concentrations were 2.5; 7.5 and 2.5 or 10.0% (Table 2). Schneider (2005) showed in her work with rose that a reduction in the sucrose concentration in the culture medium up to 10 g L^{-1} is favorable to the micropropagation of rose. These data agree with those founded in this work.

Table 2. Duncan's test to the seeds germination of Mandacaru (*Cereus jamacaru* P. DC) with different sucrose concentrations in each sodium hypochlorite concentrations.

Sodium hypochlorite (%)	Sucrose ($g.L^{-1}$)	Germination (%)
0	0	40.0 a
0	2.5	40.0 a
0	5.0	60.0 a
0	7.5	40.0 a
0	10.0	40.0 a
Means followed by the same letter, in the vertical, do not differ from each other by Duncan's (α<0.05)		
0.5	0	20.0 bc
0.5	2.5	60.0 a
0.5	5.0	0.00 c
0.5	7.5	40.0 ab
0.5	10.0	60.0 a
Means followed by the same letter, in the vertical, do not differ from each other Duncan's (α<0.05)		
1.0	0	40.0 ab
1.0	2.5	0.00 c
1.0	5.0	0.00 c
1.0	7.5	60.0 a
1.0	10.0	20.0 bc
Means followed by the same letter, in the vertical, do not differ from each other by Duncan's (α<0.05)		
1.5	0	20,0 b
1.5	2.5	60.0 a
1.5	5.0	40.0 ab
1.5	7.5	20.0 b
1.5	10.0	60.0 a
Means followed by the same letter, in the vertical, do not differ from each other by Duncan's (α<0.05)		
2.0	0	0.00 a
2.0	2.5	0.00 a
2.0	5.0	0.00 a
2.0	7.5	0.00 a
2.0	10.0	0.00 a

When the hypochlorite was used at 2% were detect phytotoxic activity with lower rates of germination. Chaves et al (2005), working with the Physalis genus demonstrated that the sodium hypochlorite at 2.5% was more efficient in disinfestations of seeds and there were no phytotoxic effects.

The best concentration of sodium hypochlorite to prevent the contamination was 1.0% (Table 3). Chalupa (1994) and Junker and Favre (1994) working with other plants species showed that the more efficient shoot disinfestations was achieved with 0.75% NaOCl solution for 10 min.

Table 3. Duncan's test to the variable contamination Mandacaru (*Cereus jamacaru* P. DC). *in vitro*, with different sodium hypochlorite concentrations.

Sodium Hypochlorite	Contamination (%)
0	20.0 bc
0.5	60.0 a
1.0	0.00 c
1.5	40.0 ab
2.0	60.0 a

Means followed by the same letter, in the vertical, do not differ from each other by Duncan's (α<0,05)

CONCLUSIONS

The sodium hypochlorite is effective to disinfestations of the Mandacaru seeds at 0.5%. The sucrose concentration more effective was 2.5% in promoting the Mandacaru's seeds germination.

ACKNOWLEDGEMENTS

The research presents in this paper was sponsored by research grants from CAPES, CNPq and FINEP. We would like to thank to Elizabel de Souza Ramalho Viana and Fernando Luiz Finger for their insightful feedback on a preliminary version of this paper.

REFERENCES

ANDRADE, C.T.S.; MARQUES, J.G.; ZAPPI, D.C. Utilização de cactáceas por sertanejos baianos. tipos conexivos para definir categorias Utilitárias. **Sitientibus Série Ciências Biológicas,** v.6, p.3-12, 2006.

BARTLETT, M.S. The saquare root transformation in analysis of variance. **Journal of statistics Society**, v**.**3, p68-78, 1936.

BRESSAN, P.H. et al. Factors affecting *in vitro* propagation of rose. **Journal of the American Society for Horticultural Science**, v.107, p.979-990, 1982.

CALDAS, L.S.; HARIDASAN, P.; FERREIRA, M.E. Meios Nutritivos. In: TORRES, A.C.; CALDAS, L.S.; BUSO, J.A. **Cultura de Tecido e Transformação Genética**. Brasília: Embrapa-CNPH, v.1, p.87-132, 1998.

CAVALCANTE, N.B.; RESENDE, G.M. Efeito de Diferentes Substratos no Desenvolvimento de Mandacaru (*Cereus jamacaru* P. DC.), Facheiro (*Pilosocereus pachycladus* RITTER), Xiquexique (*Pilosocereus gounellei* (A. WEBWR EX K. SCHUM.) BLY. EX ROWL.) e Coroa-de-Frade (*Melocactus bahiensis* BRITTON & ROSE). **Revista Caatinga**, v.20, n.1, p.28-35, 2007.

CHALUPA, V. *In vitro* propagation UNCKER, B.; FAVRE, J. M. Long-term effects of culture establishment from shoot-tip explants in micropropagation oak (*Quercus robur* L.) **Annales des Sciences Forestières**, v.51, n.6, p.581-588, 1994.

CHAVES, A.C.; SCHUCH, M.W.; ERIG, A.C. *In Vitro* establishment and multiplication of *Physalis peruviana* L. **Ciência e Agrotecnologia**, v. 29, n.6, p.1281-1287, 2005.

CRUZ, C.D. **Programa GENES (versão Windows), aplicativo computacional em genética e estatística**. Viçosa: UFV, 2001. 642p.

FOWLER, M. R. Plant cell culture, laboratory techniques. In: SPIER, R. E **Encyclopedia of Cell Technology.** New York: John Wiley & Sons, 2000. p.994-1002.

MALDANER, J. et al. Sacarose e nitrogênio na multiplicação de *Pfaffia glomerata* (spreng) Pedersen. **Ciência Rural**, v.6, n.4, p.1201-1206, 2006.