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In vitro germination and disinfestation of sweet cactus (*Nopalea cochenillifera* (L.) Salm Dyck)

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ABSTRACT. This work aimed to promote *in vitro* seed germination and disinfestation of the sweet cactus. Seeds were submerged in alcohol at 70% for 1 min. and then treated with sodium hypochlorite solution at different concentrations: 0.0; 0.5; 1.0 and 1.5%. After this treatment, the seeds were washed four times in distillated, deionized and autoclaved water. Seeds were inoculated in MS medium with different concentrations of sucrose (0.0, 2.5, 5.0, 7.5 and 10.0 g L^{-1}). The experimental design was completely randomized in a factorial experiment 4 x 5, with five replicates for treatments. Contamination, germination and seedling growth were evaluated. The results of the analysis of variance indicate that there were no significant interactions among factors. There was no significance for sucrose concentrations on contamination but these treatments showed significant differences for seed germination and seedling growth ($p \leq 0.01$). At higher sucrose concentrations there was lower germination and smaller seedlings. There were significant differences between the treatments with sodium hypochlorite for all traits analysed ($p \leq 0.01$). There was low contamination with the increase in sodium hypochlorite concentrations with higher germination percentage and taller seedlings.

Keywords: Cactaceae, forage, tissue culture.

RESUMO. Desinfestação e germinação de sementes de palma doce (*Nopalea cochenillifera* (L.) Salm Dyck) *in vitro*. Este trabalho objetivou desinfestar e promover a germinação de sementes de palma doce "*in vitro*". As sementes foram mergulhadas em álcool a 70% por 1 min. e, posteriormente, imersas em solução de hipoclorito de sódio nas concentrações de 0,0; 0,5; 1,0 e 1,5% com três gotas de Tween 20 por 10 min. e lavadas em quatro águas bidestiladas estéreis. As sementes foram cultivadas em meio MS suplementado com 0,0; 2,5; 5,0; 7,5 e 10,0% de sacarose. O delineamento experimental foi inteiramente casualizado, em esquema fatorial 4 x 5 (quatro concentrações de hipoclorito de sódio e cinco concentrações de sacarose), com cinco repetições. Avaliou-se a contaminação, germinação e crescimento da plântula. Não houve interação significativa entre os fatores, havendo porém, diferenças significativas entre os tratamentos de hipoclorito e concentrações de sacarose ($p \leq 0,01$). As concentrações de sacarose não tiveram efeito significativo na contaminação do meio, porém com o aumento dos teores de sacarose houve tendência ao decréscimo na germinação e crescimento da plântula ($p \leq 0,01$). Quanto maior a concentração de hipoclorito no meio menor foi a contaminação e maior a germinação e comprimento da plântula ($p \leq 0,01$).

Palavras-chave: Cactaceae, forrageira, cultura de tecido.

Introduction

Nopalea cochenilifera (L.) Salm Dyck is known in Brazil as Small Palm or Sweet Palm and is cultivated in the Brazilian semi-arid northeast, mainly by the dairy cattle ranchers, and the largest crop areas are found in the States of Alagoas, Pernambuco and Paraíba. It has been used as a food supply, in the drought period by small farmers (MAIA NETO, 2003).

In long-term drought, the ranchers cultivate cacti due to its high water content vital to livestock rising, and ranchers feed them through all year round, as in the same way as *Opuntia ficus-indica* (SANTOS et al., 1996).

Maintenance collections of succulent plants can be problematic since many of theses species are very susceptible to rots by bacteria and fungi. Although their seeds could be storaged at low relative humidity and temperature, these microoorganisms

can affect the seeds germination vigor and can promote the anormal growing of plantlets in tissue culture. Methods for seed disinfestation in micropropagation of cacti have been developed to overcoming these problems and to obtain healthy plants (COUTO et al., 2004).

Disinfestations of *Opuntia* seeds are necessary when the germination is performed *in vitro*. In such case, cultures are supplemented by a source of sugar and salts in order to obtain energy and other requirements. The nutritional requirements by explants *in vitro* depend on the species, varieties and tissue (CALDAS et al., 1998). Reis et al. (2009) showed the influence of the culture medium in the content and chemical composition of the essential oil of *Melissa officinalis*. Sucrose is included in culture media to promote rapid growth (CALDAS et al., 1998; MALDANER et al., 2006). In tissue culture, the use of sugar is necessary, sometimes due to the low photosynthetic rate as presented by orchids and cactus. However, the presence of sugar and salts easily allows the development of unwanted microorganisms. To avoid such phenomenon, an efficient procedure must be done as a pretreatment of the seeds with liquid or gaseous substances (ALVAREZ-PARDO et al., 2006).

The liquid disinfectant should remove or kill all microorganisms from the seeds' surface without damaging the embryos. For the purpose of disinfesting seeds, the calcium hypochlorite is the most used one, besides hydrogen peroxide and sodium hypochlorite. Sodium and calcium hypochlorite are prescribed to be used at different concentrations and times of exposure to disinfest seeds (ARDITTI; ERNST, 1984; PIERRIK, 1997).

The present investigation was carried out with the objectives to promote *in vitro* seed germination, disinfestation and plant growth of the sweet cactus.

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; followed rinsing with sterile deionized water and dipped in a commercial sodium hypochlorite solution at different concentrations: 0.0; 0.5; 1.0 and 1.5% for 10 minutes, established based on previous experiment. After this treatment, the seeds were washed 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. 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 during 15 days at 25±2°C and then moved to a room with 16 hours of light exposure with a luminosity of 30 μmol m^{-2} s and 8 hours under darkness. The swollen seeds with green embryos were observed with a stereomicroscope and they 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 4 x 5, with five replicates for treatments. The following variables were evaluated: contamination, germination seed frequency and seedling growth. The results were submitted to statistical ANOVA analysis and followed by regression analysis ($p \leq 0.01$). The statistical analyses were done using Genes software (CRUZ, 2001).

Results and discussion

The results of ANOVA showed that there was no significantly interaction between sucrose concentrations and sodium hypochlorite concentrations (Table 1). This indicates that the factors affect the response independently and should be analyzed separately. Thus, the regressions analysis for all treatments in a factor should be examined as resulted from a single-factor experiment (Figures 1 and 2).

There were significant differences between the treatments with sodium hypochlorite for all traits analyzed ($p \leq 0.01$) (Table 1). The disinfestations treatments 0.5; 1.0 and 1.5% were effective in to control the contamination. The germination

increased with the desinfestation with sodium hypochlorite and started at seventh day after the inoculation of the seeds (Figure 1).

Table 1. Analysis of variance of contamination, germination and seedling growth in Sweet cactus (*Nopalea cochenillifera*) *in vitro*.

Source of variation	D.F.	Mean Square		
		Contamination	Germination	Seedling growth
Sucrose (S)	4	0.004^{ns}	0.258**	32.43**
Sodium Hipoclorite (NaHClO)	3	1.424**	0.338**	21,58**
(NaHClO) x S	12	0.01^{ns}	0.037^{ns}	3.453^{ns}
Residue	80	0.01	0.042	3.944
Total	99			

**Significant ($p \leq 0.01$) by F statistics; ^{ns}no significant.

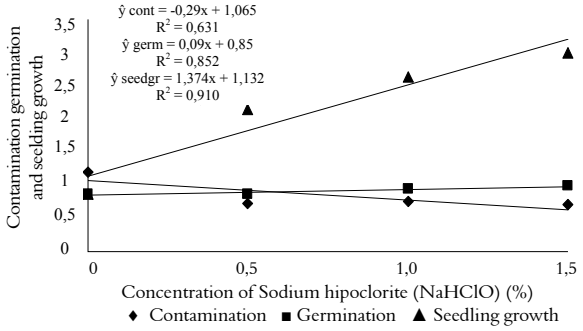

Figure 1. Regression analysis of contamination, germination and seedling growth in Sweet cactus (*Nopalea cochenillifera*) *in vitro*, with different sodium hypochlorite concentrations.

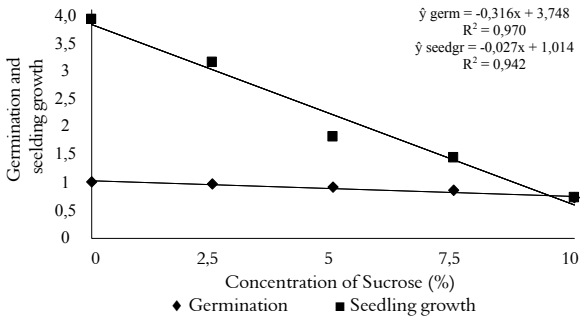

Figura 2. Regression analysis of germination and seedling growth in Sweet cactus (*Nopalea cochenillifera) in vitro*, with different sucrose concentrations.

We verified a higher germination percentage and taller seedlings with the increase in sodium hypochlorite concentrations (Figure 1). The beginning of contamination was detected at third day after the inoculation of seeds. According to Barrueto Cid and Zimmermann (2006), the sodium hypochlorite is the most efficient product on disinfestation of explants. Golle et al. (2010) working with *Pinus taeda* L. seeds and using sodium hypochlorite at several concentrations in different periods for *in vitro* disinfestation founded the combination of NaOCl at 3% for 5 minutes provided a good disinfestation but inhibited the seeds germination.

There was no significance for sucrose concentrations on contamination but these treatments showed significant differences for seed germination and seedling growth ($p \leq 0.01$) (Table 1). The germination and the seedling growth decreased with the increase in sucrose concentration (Figure 2). The same was observed by Leal et al. (2005), studying the germination and development of *Pitaya* with different concentrations of sucrose. Nevertheless, Dávila-Figueroa et al. (2005) observed significant effects of 3% sucrose for *in vitro* propagation of *Turbinicarpo*. Unlike, Da Luz et al. (2008) found no differences for *in vitro* polen germination using differents sucroses concentration.

Conclusion

Sodium hypochlorite was able to decrease the contamination in sweet cactus *in vitro* germination, and seedling growth; and the higher sucrose concentration reduced the seed germination and seedling growth. Data are also presented regarding the disinfestation, and asymbiotic seed germination represents an efficient way to propagate this species.

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