In vitro germination and acclimatization of *Hamatocactus setispinus*[1]

PRISCILLA BRITES XAVIER[2]* and JANIE MENDES JASMIM[2]

ABSTRACT

Seed propagation preserves the population genetic variability and helps selecting desirable features. This study evaluated the in vitro germination of *Hamatocactus setispinus* in six different culture media, 1- MS basal medium full strength; 2- half-strength MS basal medium; 3- 1.0 g L^{-1} of Peter's CalMag® 15-05-15 formulation; 4- 0.5 g L^{-1} of Peter's CalMag® 15-05-15 formulation; 5- MS basal medium supplemented with 10% coconut water and; 6- water and agar, with and without activated charcoal, and the speed of germination index, the mean germination time and the germination rate, root length, shoot length and the number of roots were evaluate. The seedlings with superior development obtained from in vitro germination were acclimatized in two substrates: Biomix® Floreira; Biomix® Floreira + sand. Seedling survival, shoot length, shoot diameter, root length, root number, shoot fresh matter weight, root fresh matter weight, shoot dry matter weight and root dry matter weight were evaluated. Peter's 1.0 g L^{-1} medium without activated charcoal led to the best results for root length (11.36 mm) and root number (3.84). There was 100% of seedling survival. Acclimatization substrates did not differ among themselves and, therefore, they did not affect seedling growth.

Keywords: *Hamatocactus setispinus*, activated charcoal, culture media, cacti.

RESUMO

G**erminação** *in vitro* **e aclimatização de** *Hamatocactus setispinus*

A propagação de sementes preserva a variabilidade genética da população e ajuda a selecionar características desejáveis. Objetivou-se estudar a germinação *in vitro* de *Hamatocactus setispinus* em seis diferentes meios de cultura, 1- meio de cultivo MS; 2- MS ½ força de sais minerais; 3- 1 g L^{-1} da formulação 15-05-15 Peter's® CalMag; 4- 0,5 g L^{-1} da formulação 15-05-15 Peter's® CalMag; 5- MS com adição de 10% de água de coco e; 6- água e ágar, com e sem carvão ativado, avaliando o índice de velocidade de germinação, o tempo médio de germinação e a taxa de germinação, comprimento da raiz, comprimento da parte aérea e o número de raízes. As plântulas com desenvolvimento superior obtidas da germinação *in vitro* foram aclimatizadas em dois substratos: Biomix® Floreira; Biomix® Floreira + areia. Foram avaliados: sobrevivência de plântulas, comprimento da parte aérea, diâmetro da parte aérea, comprimento da raiz, número de raízes, matéria fresca da parte aérea, matéria fresca da raiz, matéria seca da parte aérea e matéria seca da raiz. O meio Peter's 1,0 g L^{-1} sem carvão ativado levou a melhores resultados no comprimento de raiz (11,36 mm) e número de raízes (3,84). Houve 100% de sobrevivência das mudas. Os substratos de aclimatação não diferiram entre si e, por isso não afetaram o crescimento das mudas.

Palavras-chave: *Hamatocactus setispinus,* carvão ativado, meio de cultura, cacto.

1. INTRODUCTION

Seed germination stands out among the methods for cacti propagation, because it allows the preservation of genetic diversity of populations (ROJAS-ARÉCHIGA and VÁSQUEZ-YANES, 2000), which might help in the selection of desirable features, such as biomass production, fruit quality, tolerance to stress-promoting factors, etc. (ALTARE et al., 2006).

Many cactus species have slow growth and low seed germination. Thus, in order to increase the production of these plants, *in vitro* propagation is an important tool (MEDEIROS et al., 2006), not only for enhancing increased growth rates, but also for producing plants free of pathogens (ROJAS-ARÉCHIGA and VÁSQUEZ-YANES, 2000).

For a successful *in vitro* cultivation, it is important to know the nutritional requirements of cells and tissues in culture. Therefore, one of the factors that affect the success of this technique is the culture medium in which the explants are grown.

Culture media are based on plant nutrient requirements and, in general, consist of essential components that comprise inorganic salts, carbon source and energy, water, vitamins and growth regulating substances. However, in order to comply with specific needs, some modifications can be made by adding optional components, such as amino acids and amides, organic acids and complex natural substances (THORPE, 1981).

After *in vitro* cultivation, plants must be acclimatized and this process should be carried out very carefully, due to differences between *in vitro* and greenhouse environmental conditions (HAZARIKA, 2003).

[1] Received in 15/08/2013 and approved in 10/06/2015.

[2] Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Centro de Ciências e Tecnologias Agropecuárias (CCTA), Laboratório de Fitotecnia (LFIT), Campos dos Goytacazes-RJ, Brasil. *Corresponding author: pri_brites@yahoo.com.br.

Thus, the objective was to evaluate the *in vitro* germination of *Hamatocactus setispinus* in different culture media, with and without the addition of activated charcoal, and seedling acclimatization in different substrates.

2. MATERIALS AND METHODS

Plant material

Seeds were obtained from fruits of *Hamatocactus setispinus*, which were harvested in June 2009, in Maricá (22° 55' 10'' S, 42° 49' 07'' W), a town in Rio de Janeiro State, Brazil. The fruits were packed in paper bags and transported to the laboratory for tests.

In vitro **germination experiment**

There were evaluated six different culture media (Medium 1 - full-strength MS basal medium; Medium 2 - half-strength MS basal medium; Medium 3 - 1.0 g L^{-1} of Peter's CalMag® 15-05-15 formulation; Medium 4 - 0.5 g L^{-1} of Peter's CalMag® 15-05-15 formulation; Medium 5 - MS basal medium supplemented with 10% coconut water; Medium 6 - water and agar), combined with two concentrations of activated charcoal (3.0 g L^{-1} and 0 g L^{-1}).

Medium 1, 2 and 5 were supplemented with sucrose (30 g L^{-1}), White's vitamins (10 mg L^{-1}), myo-inositol (0.1 g L^{-1}) and Vetec® agar (8 g L^{-1}). Media 3 and 4 were supplemented only with sucrose (30 g L^{-1}) and Vetec® agar (8 g L^{-1}). Medium 6 was supplemented only with Vetec® agar (8 g L^{-1}).

Culture jars containing 30 mL of medium were autoclaved at 121° C and 1.5 atm for 15 minutes. Coconut water used to prepare Medium 5, which was extracted from a fresh green coconut, was initially filtered through cotton and, then, in filter paper to remove impurities. In a laminar flow chamber, the coconut water was filtered through a Millipore filter of 0.22 mm and then added to the respective culture medium that had already been autoclaved.

Six days after harvest, the fruits were opened and their seeds were carefully removed and spread on Kraft paper for the elimination of the mucilage. Subsequently, seeds were disinfested in a solution of 0.50% of sodium hypochlorite under constant shaking for 15 minutes in a laminar flow chamber environment. Seeds were then triple washed in deionized autoclaved water for 5, 10 and 10 minutes each. Then the seeds were inoculated in the culture jars containing 30 mL of the culture media described above, sealed with plastic caps and wrapped in PVC film.

The jars containing the seeds were kept in a culture room with fluorescent daylight-type lights of 40 W, light intensity of 25 mmol m^{-2} s^{-1}, at a temperature of 27 ± of 2° C and a photoperiod of 16 hours.

Germination was evaluated every three days during a period of 60 days. Seeds were considered germinated at radicule emission. The germination rate was evaluated and mean germination time (MT) calculated according to Edmond and Drapala (1958), whereas the speed germination index (SGI) was calculated by the formula suggested by Maguire (1962).

For each seedling, the number of roots (NR) was counted and root (RL) and shoot length (SL) were measured with a digital caliper. The experiment consisted of a 6x2 factorial scheme six different culture media and two concentrations of activated charcoal in a completely randomized design with ten replications. Each replication consisted of one culture jar containing 10 seeds, totalizing 120 jars and 1200 seeds. The germination rate data were transformed to arcsine $(x/100)^{½}$. The data were subjected to variance analysis and means were compared by Tukey test at 5% probability with the help of Genes program (CRUZ, 2001).

Acclimatization experiment

Four acclimatization experiments were established with seedlings from *in vitro* germination, in a greenhouse covered with white polyethylene film (100 m) and a 70% shade cloth (Sombrite®). The experimental designs were in randomized blocks with two substrate treatments (S1- Biomix Floreira®; S2- Biomix Floreira® + sand).

The first experiment was carried out with seedlings obtained from seed germination in Peter's® CalMag 1.0 g L^{-1} medium (Medium 3) without activated charcoal, with three replications with two pots per plot, containing 11 seedlings per pot, totalizing 66 seedlings. The second one used seedlings from seed germination in Peter's® CalMag 1.0 g L^{-1} medium (Medium 3) with activated charcoal, with three replications and two pots per plot, each containing 12 seedlings, in a total of 72 seedlings. The third one used seedlings from seed germination in Peter's® 0.5 g L^{-1} medium (Medium 4) without activated charcoal with three replications and two pots per plot, each containing 12 seedlings, in a total of 72 seedlings. The fourth and last experiment used the seedlings obtained from seed germination in Peter's® 0.5 g L^{-1} medium (Medium 4) with activated charcoal and consisted of three replications with two pots per plot, each containing 11 seedlings, totalizing 66 seedlings.

Seedlings were removed from the culture medium and the excess of medium was washed from the roots before transplanting to plastic pots (0.5 L) containing Biomix Floreira® (S1) or Biomix Floreira® + sand (S2) as substrate. The pots were kept in the greenhouse for three months, during which the average minimum and maximum temperatures registered were 24.3 ± 1.3° C, and 29.5 ± 1.2° C, respectively; the average air relative humidity was 63 ± 2.3% with a photosynthetically active photon flow of 150.8 ± 31µmol fotons m^{-2} s^{-1}.

At the end of the experimental period were evaluated: seedling survival (S%), shoot length (SL) and shoot diameter (SD), root length (RL), number of roots (NR), shoot fresh and dry weights (SFW, SDW) and root fresh and dry weights (RFW, RDW). Shoot length was measured from bottom to top of the stem; shoot diameter was measured in the middle portion of the stem (most uniform and juicy). Roots and shoots were separated for fresh weight measurements. After that, roots and shoots were dried at 70º C for 72 hours in a convection oven before dry weight measurements.

Data were subjected to joint variance analysis and means were compared by Tukey test at 5% of probability, with the help of the program SAEG (SAEG, 2007).

3. RESULTS AND DISCUSSION

In vitro **germination experiment**

Seed germination began on the sixth day after *in vitro* sowing. It could be considered fast based on data for germination of eight cacti species of the genus *Turbinicarpus*, whose onset of *in vitro* germination was on the fourteenth day (DÁVILA-FIGUEROA et al., 2005) and on similar results observed for *Pilosocereus robinii* (QUIALA et al. 2009), whereas *Arrojadoa* spp (cactus foxtail) germination began on the eighth day after sowing (DIAS et al., 2008).

For speed germination index (SGI), the best results were obtained with Peter's 1.0 g L^{-1} (Medium 3) or water and agar (Medium 6) media, without the addition of activated charcoal, obtaining 0.91 and 0.96 seed germinated per day, respectively. The lowest values of SGI were obtained with MS medium (Medium 1) and MS supplemented with 10% coconut water (Medium 5), regardless of the addition of charcoal (Table 1).

Table 1. Speed germination index (SGI), mean germination time (MT), germination percentage of *Hamatocactus setispinus* seeds obtained from *in vitro* germination in culture media: (1) MS basal medium full strength, (2) half-strength MS basal medium, (3) 1.0 g L^{-1} of Peter's CalMag® 15-05-15 formulation, (4) 0.5 g L^{-1} of Peter's CalMag® 15-05-15 formulation, (5) MS basal medium supplemented with 10% coconut water, (6) water and agar, without (0 g L^{-1}) and with (3 g L^{-1}) activated charcoal.

Tabela 1. *Índice de velocidade de germinação (IVG), tempo médio de germinação (Tm), percentual de germinação de sementes de Hamatocactus setispinus obtidas a partir de germinação in vitro em meios de cultivo MS (1), MS ½ força de sais minerais (2), 1.0 g L^{-1} da formulação 15-05-15 Peter's® CalMag (3), 0,5 g L^{-1} da formulação 15-05-15 Peter's® CalMag (4), MS com adição de 10% de água de coco (5) e água e ágar (6), sem (0 g L^{-1}) e com (3 g L^{-1}) carvão ativado.*

	SGI			MT			Germination (%)		
Culture media	Without activated chacoal	With activated chacoal	Mean	Without activated chacoal	With activated chacoal	Mean	Without activated chacoal	With activated chacoal	Mean
1	0.34 Ca	0.35 CDa	0.34	19.37 Ba	15.33 BCb	17.35	58 Ba	44 Ba	50
2	0.57 Ba	0.53 BCa	0.55	15.62 BCa	18.16 Ba	16.89	76 ABa	74 Aa	76
3	0.91 Aa	0.77 Ab	0.84	12.67 Ca	11.42 Ca	12.04	94 Aa	74 Aa	85
4	0.51 BCb	0.77 Aa	0.64	18.74 Ba	13.83 BCb	16.28	79 ABa	77 Aa	77
5	0.34 Ca	0.31 Da	0.33	27.67 Aa	25.66 Aa	26.67	79 ABa	69 Aba	74
6	0.96 Aa	0.64 ABb	0.80	11.07 Ca	14.34 BCa	12.71	90 Aa	79 Aa	85
Mean	0.61	0.56	0.58	17.52	16.46	16.99	79	69	74
CV (%)	25.31			23.14			15.42		

Upper-case letters compare culture media and lower-case letters compare the addition of activated charcoal. Means followed by the same letters do not differ significantly at 5% probability by Tukey test.

The lowest mean germination times (MT) were observed in Medium 3 and Medium 6 without activated charcoal, with means of 12.67 and 11.07 days, respectively. The highest values of MT were obtained with Medium 5, regardless of the use of charcoal, with means of 27.67 and 25.66 days, respectively, with and without charcoal. In general, the use of charcoal did not affect this characteristic, a result different from that reported by Dias et al. (2008), where the lowest values of MT were observed in media supplemented with activated charcoal.

For seed germination rate, no significant differences were observed among media or between activated charcoal treatments, except for Medium 1 that showed the lowest values either with or without charcoal (Table 1). Rosas-López and Collazo-Ortega (2004) evaluating three different culture media on the germination of *Polaskia chichipe* and *Echinocactus platyacanthus* (water and agar, half-strength MS basal medium and; full-strength MS basal medium) also observed the lowest germination rate in MS medium with 100% of minerals. Such results might be due to the concentration of salts decreasing the water potential in the medium, which would consequently reduce the water availability necessary for germination (ROSAS-LÓPEZ and COLLAZO-ORTEGA, 2004). According to the same authors, the medium consisting of water and agar not only had a higher germination rate, but also a higher SGI due to higher water potential of the medium when compared to the other media used. The same relationship between the concentration of salts in the medium and its water potential was discussed by Santos (2009).

The use of activated charcoal in the medium led to the highest values in shoot length (SL) (Table 2). A similar result was observed by Bellintani et al. (2007) for two species of bromeliads, where plants from the medium with charcoal had greater values of shoot length, which might be related to the adsorption of undesirable substances by the charcoal. According to Hartmann et al. (2002) one of the advantages of activated charcoal is its ability to adsorb substances secreted by explants, or present in the culture medium, which can inhibit growth.

Table 2. Shoot length (SL), root length (RL) and root number (RN) of *Hamatocactus setispinus* seedlings obtained from *in vitro* germination in culture media: (1) MS basal medium full strength, (2) half-strength MS basal medium, (3) 1.0 g L^{-1} of Peter's CalMag® 15-05-15 formulation, (4) 0.5 g L^{-1} of Peter's CalMag® 15-05-15 formulation, (5) MS basal medium supplemented with 10% coconut water, (6) water and agar, without (0 g L^{-1}) and with (3 g L^{-1}) activated charcoal.
Tabela 2. *Comprimento da parte aérea (CPA), comprimento de raiz (CR) e número de raízes (NR) de plântulas de Hamatocactus setispinus obtidas a partir de germinação in vitro em meios de cultivo MS (1), MS ½ força de sais minerais (2), 1.0 g L^{-1} da formulação 15-05-15 Peter's® CalMag (3), 0,5 g L^{-1} da formulação 15-05-15 Peter's® CalMag (4), MS com adição de 10% de água de coco (5) e água e ágar (6), sem (0 g L^{-1}) e com (3.0 g L^{-1}) carvão ativado.*

	SL (mm)			RL (mm)			RN		
Culture media	Without activated chacoal	With Activated charcoal	Mean	Without activated chacoal	With Activated charcoal	Mean	Without activated chacoal	With Activated charcoal	Mean
1	4.04 Cb	7.05 Ca	5.55	6.21 Ca	5.09 CDa	5.65	1.72 CDa	1.78 Ba	1.75
2	4.23 Cb	9.78 ABa	7.00	7.48 BCa	8.39 Aba	7.93	2.26 BCb	2.85 Aa	2.56
3	7.70 Ab	11.24 Aa	9.47	11.36 Aa	8.99 Ab	10.18	3.84 Aa	3.36 Ab	3.60
4	6.06 ABb	9.32 Ba	7.69	9.51 ABa	7.23 ABCb	8.37	2.88 Ba	3.10 Aa	2.99
5	4.42 BCb	9.23 Ba	6.82	8.28 BCa	6.09 BCb	7.18	1.84 CDa	1.49 BCa	1.67
6	3.50 Cb	5.37 Da	4.43	3.45 Da	2.89 Da	3.17	1.30 Da	1.02 Ca	1.16
Mean	4.99	8.67	6.83	7.71	6.45	7.08	2.31	2.27	2.29
CV (%)	18.95			29.47			22.48		

Upper-case letters compare culture media and lower-case letters compare the addition of activated charcoal. Means followed by the same letters do not differ significantly at 5% probability by Tukey test.

Medium 3 led to the highest SL values, while the seedlings on Medium 6 had the lowest ones (Table 2), probably this medium did not provide enough nutrients for normal seedling growth. The presence of water just accelerated seed germination, but for seedling continued development nutrients from the culture medium would be required.

In a study with seeds of *Cattleya loddigessi*, Moraes et al. (2009) also obtained higher root length and number of roots on media supplemented with commercial soluble formulation of minerals. They observed highest values of seedling root length on culture medium supplemented with orange Kristalon fertilizer (NPK: 6-12-36) and best results for root number on culture medium supplemented with Hyponex fertilizer (NPK: 6.5-6-19), when compared to results obtained on mineral half strength MS medium.

Table 3. Shoot length (SL) and shoot diameter (SD) of *Hamatocactus setispinus* seedlings obtained from *in vitro* germination in culture media Peter´s 1.0 g L^{-1} and Peter´s 0.5 g L^{-1} without (0 g L^{-1}) and with (3.0 g L^{-1}) activated charcoal after an acclimatization period of three months in Biomix Floreira® (S1) and Biomix Floreira® + sand (S2) acclimatization substrates.
Tabela 3. *Comprimento da parte aérea (CPA) e diâmetro da parte aérea (DPA) de plântulas de Hamatocactus setipinus obtidas a partir de germinação in vitro nos meios de cultivo Peter´s 1.0 g L^{-1} e Peter´s 0,5 g L^{-1} sem (0 g L^{-1}) e com (3.0 g L^{-1}) carvão ativado após três meses de aclimatização nos substratos Biomix Floreira® (S1) e Biomix Floreira® + areia (S2).*

Germination culture media	Activated charcoal	SL (mm)			SD (mm)		
		Substrate			Substrate		
		S1	S2	Mean	S1	S2	Mean
Peter's 1.0 g L^{-1}	Without	21.94 Bb	23. 27 Ba	22.61	13.64 Ba	14.29 Aa	13.97
	With	26.12 Aa	26.67 Aa	26.40	15.33 Aa	15.59 Aa	15.46
Peter's 0.5 g L^{-1}	Without	19.07 Ca	20.06 Ca	19.57	12.00 Ca	12.16 Ba	12.08
	With	23.12 Bb	25.66 Aa	24.39	14.65 ABa	15.39 Aa	15.02
Mean		22.56	23.91	23.24	13.91	14.36	14.14
CV (%)		2.24			3.86		

Upper-case letters compare culture media and lower-case letters compare substrates. Means followed by the same letters do not differ significantly at 5% probability by Tukey test.

Acclimatization experiment

The seedling survival was 100% in all four acclimatization experiments. There was no significant difference in SD (Table 3), RN (Table 4), SFW, RFW (Table 5), SDM and RDM (Table 6) of seedlings from either substrate on the four acclimatization experiments.

There was a significant interaction between substrate x *in vitro* culture medium for shoot (SL) (Table 3) and root length (RL) (Table 4). Seedlings from Peter's® CalMag 1.0 g L^{-1} culture medium with activated charcoal had the highest SL, when grown in Biomix® Floreira (S1), whereas those from Peter's® 1.0 and 0.5 g L^{-1} culture media with activated charcoal had the highest SL (Table 3). Moreover, Peter's® 0.5 g L^{-1} medium without activated charcoal led to the lowest values of SL for plants grown in both substrates. Seedlings grown in Biomix® Floreira + sand (S2), originally obtained from Peter's® 1.0 g L^{-1} without charcoal and Peter's® 0.5 g L^{-1} with charcoal *in vitro* culture media, showed the highest values of SL. Seedlings, originally obtained from other *in vitro* culture media, did not differ in SL values regardless of the acclimatization substrate used.

Table 4. Root length (RL) and root number (RN) of *Hamatocactus setispinus* seedlings obtained from *in vitro* germination in culture media Peter´s 1.0 g L^{-1} and Peter´s 0.5 g L^{-1} without (0 g L^{-1}) and with (3.0 g L^{-1}) activated charcoal after an acclimatization period of three months in Biomix Floreira® (S1) and Biomix Floreira® + sand (S2) acclimatization substrates.

Tabela 4. *Comprimento da raiz (CR) e número de raízes (NR) de plântulas de Hamatocactus setipinus obtidas a partir de germinação in vitro nos meios de cultivo Peter´s 1.0 g L^{-1} e Peter´s 0,5 g L^{-1} sem (0 g L^{-1}) e com (3.0 g L^{-1}) carvão ativado após três meses de aclimatização nos substratos Biomix Floreira® (S1) e Biomix Floreira® + areia (S2).*

Germination culture media	Activated charcoal	RL (mm)			RN		
		Substrate			Substrate		
		S1	S2	Mean	S1	S2	Mean
Peter's 1 g L^{-1}	Without	32.32 Aa	35.89 ABa	34.11	3.64 Aa	3.70 Aa	3.67
	With	29.76 Ab	39.46 Aa	34.61	2.53 Ba	2.44 Ba	2.49
Peter's 0.5 g L^{-1}	Without	27.17 Aa	28.39 Ba	27.78	2.08 Ca	2.14 Ba	2.11
	With	33.78 Aa	33.66 ABa	33.72	2.30 BCa	2.43 Ba	2.37
Mean		30.76	34.35	32.56	2.64	2.68	2.66
CV (%)		11.96			4.93		

Upper-case letters compare culture media and lower-case letters compare substrates. Means followed by the same letters do not differ significantly at 5% probability by Tukey test.

Table 5. Shoot (SFW) and root (RFW) fresh weights of *Hamatocactus setispinus* seedlings obtained from *in vitro* germination in culture media Peter´s 1.0 g L^{-1} and Peter´s 0.5 g L^{-1} without (0 g L^{-1}) and with (3.0 g L^{-1}) activated charcoal after an acclimatization period of three months in Biomix Floreira® (S1) and Biomix Floreira® + sand (S2) acclimatization substrates.

Tabela 5. *Matéria fresca de parte aérea (MFPA) e matéria fresca de raiz (MFR) de plântulas de Hamatocactus setipinus obtidas a partir de germinação in vitro nos meios de cultivo Peter´s 1.0 g L^{-1} e Peter´s 0,5 g L^{-1} sem (0 g L^{-1}) e com (3.0 g L^{-1}) carvão ativado após três meses de aclimatização nos substratos Biomix Floreira® (S1) e Biomix Floreira® + areia (S2).*

Germination culture media	Activated charcoal	SFW (g)			RFW (g)		
		Substrate			Substrate		
		S1	S2	Mean	S1	S2	Mean
Peter's 1.0 g L^{-1}	Without	1.45 BCa	1.62 Ba	1.54	0.0238 Aa	0.0332 Aa	0.0285
	With	2.06 Aa	2.07 Aa	2.07	0.0174 Aa	0.0194 ABa	0.0184
Peter's 0.5 g L^{-1}	Without	1.11 Ca	1.14 Ca	1.23	0.0118 Aa	0.0101 Ba	0.0110
	With	1.73 ABa	1.93 ABa	1.83	0.0176 Aa	0.0135 Ba	0.0156
Mean		1.59	1.69	1.64	0.0177	0.0191	0.0184
CV (%)		9.27			33.42		

Upper-case letters compare culture media and lower-case letters compare substrates. Means followed by the same letters do not differ significantly at 5% probability by Tukey test.

Seedlings grown in S1, originally obtained from Peter's® 1.0 and 0.5 g L^{-1} *in vitro* culture medium with charcoal, showed the highest SD values (15.33 and 14.65 mm, respectively), while those from Peter's® 0.5 g L^{-1} medium without charcoal showed the lowest ones (12.00 mm) (Table 3). In acclimatization substrate S2, seedlings originally from Peter's® 0.5 g L^{-1} medium without charcoal also had the lowest SD values, whereas seedlings from other culture media showed higher SD values and did not differ among themselves.

Seedling RL did not differ according to their original culture media, when plants were grown in acclimatization substrate S1. Nevertheless, when acclimatized in substrate S2, seedlings obtained from Peter's® 1.0 g L^{-1} medium with charcoal had longer RL than those originally from Peter's® 0.5 g L^{-1} medium without charcoal (Table 3). Seedlings originally from Peter's® 1.0 g L^{-1} culture medium without charcoal showed higher values of RN in both acclimatization substrates (Table 4) compared to seedlings from the other culture media.

The SFW values of seedlings from Peter's® 1 and 0.5 g L^{-1} media with charcoal grown in both acclimatization substrates did not differ significantly among themselves and were higher than those of seedlings from the other *in vitro* culture media, but SFW values of those from Peter's® 0.5 g L^{-1} without charcoal were the lowest ones (Table 5).

There was no significant difference among the effects of the *in vitro* culture media on the seedling RFW, when they were grown in acclimatization substrate S1, whereas those grown in substrate S2, originally from Peter's® 1.0 g L^{-1} medium (either with or without charcoal), showed the highest values for this trait (Table 5).

There was no significant difference between acclimatization substrate effects on SDM and RDM, irrespective of the seedling original culture medium (Table 6).

In the *in vitro* germination experiment, a detrimental effect of activated charcoal on germination was observed (Table 1). This result contrasts that observed in the acclimatization experiments in which the seedlings from media containing charcoal either did not differ or showed superior results, for some characteristics, than seedlings originally from media without charcoal. Thus, envisaging the large scale *in vitro* production of seedlings, the use of activated charcoal could be recommended for this cactus species. Nevertheless, considering the seedling acclimatization results, it would be advisable to further investigate extended acclimatization periods for a conclusive recommendation based on the presence of activated charcoal in the germination culture medium and its effect on the seedling growth during acclimatization.

Table 6. Shoot dry (SDW) and root dry (RDW) weights of *Hamatocactus setispinus* seedlings obtained from *in vitro* germination in culture media Peter´s 1.0 g L^{-1} and Peter´s 0.5 g L^{-1} without (0 g L^{-1}) and with (3.0 g L^{-1}) activated charcoal after an acclimatization period of three months in Biomix Floreira® (S1) and Biomix Floreira® + sand (S2) acclimatization substrates.

Tabela 6. *Matéria seca da parte aérea (MSPA) e matéria seca de raiz (MSR) de plântulas de Hamatocactus setipinus obtidas a partir de germinação in vitro nos meios de cultivo Peter´s 1.0 g L^{-1} e Peter´s 0,5 g L^{-1} sem (0 g L^{-1}) e com (3.0 g L^{-1}) carvão ativado após três meses de aclimatização nos substratos Biomix Floreira® (S1) e Biomix Floreira® + areia (S2).*

Germination culture media	Activated charcoal	SDW (g)			RDW (g)		
		Substrate			Substrate		
		S1	S2	Mean	S1	S2	Mean
Peter's 1.0 g L^{-1}	Without	0.0474 Aa	0.0556 Aa	0.0515	0.0036 Aa	0.0053 Aa	0.0045
	With	0.0657 Aa	0.0613 Aa	0.0635	0.0035 Aa	0.0045 Aa	0.0040
Peter's 0.5 g L^{-1}	Without	0.0555 Aa	0.0398 Aa	0.0477	0.0036 Aa	0.0026 Aa	0.0031
	With	0.0569 Aa	0.0675 Aa	0.0622	0.0033 Aa	0.0043 Aa	0.0038
Mean		0.0564	0.0561	0.0563	0.0035	0.0042	0.0039
CV (%)		26.08			35.40		

Upper-case letters compare culture media and lower-case letters compare substrates. Means followed by the same letters do not differ significantly at 5% probability by Turkey Test

4. CONCLUSIONS

The culture medium consisting of 1.0 g L^{-1} Peter's CalMag (15-05-15) supplemented with sucrose (30 g L^{-1}), Vetec® agar (8 g L^{-1}) and activated charcoal (3 g L^{-1}) showed the best results for *in vitro* germination of *Hamatocactus setispinus*.

Substrates Biomix Floreira® (S1) and Biomix Floreira® + sand (S2) can be used for acclimatization of *in vitro* produced seedlings of this cactus species.

ACKNOWLEDGEMENTS

To FAPERJ for the research grants, Botanica Pop Ltda for the cactus seeds, Setor de Cultura de Tecidos of the Laboratorio de Fitotecnia of the Universidade Estadual do Norte Fluminense Darcy Ribeiro for the *in vitro* experiment.

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