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BIOLOGY

Development of the prickly pear cactus *Opuntia stricta* (Haw.) Haw. (Cactaceae) in vitro in response to the replacement of potassium nitrate for a commercial kno_3 fertilizer

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ABSTRACT: *In micropropagation, potassium nitrate (KNO_3) an ACS reagent grade chemical, used in the preparation of growing mediums is expensive and its procurement depends on bureaucratic procedures, as it is controlled by the Brazilian Army. This research to assessed the effect of replacing the ACS KNO_3 for a commercially available fertilizer (KNO_3- based) on the micropropagation of the prickly pear cactus (Opuntia stricta (Haw.) Haw. cv. Elephant Ear. Treatments used six different fertilizer concentrations (0, 0.5, 1, 1.5, 2 and 2.5 g L^{-1}) and a control consisting of 1.9 g L^{-1} KNO_3, as shown in the MS salts. The survival, size and number of sprouts and the value of fresh biomass were evaluated. After seedling acclimation, we assessed the survival, number of sprouts, length, and number of roots, racket formation, average fresh biomass mass, macronutrient absorption and morphological changes of the seedlings. Explants inoculated with fertilizers at concentrations of 0.0; 2.0 and 2.5 g L^{-1} did not grow. The response of explants at concentrations of 0.5 and 1.5 g L^{-1} of the fertilizer were the same as those developed in a KNO_3 medium, and at a concentration of 1.0 g L^{-1}, in all variables, the means were higher than those of the control medium. Therefore, it showed the feasibility of using fertilizers in the in vitro cultivation of the prickly pear cactus, which may remove bureaucratic barriers and reduce product costs by 99.12%.*
Key words: *macronutrients, micropropagation, cost savings.*

Desenvolvimento da palma *Opuntia stricta* (Haw.) Haw. (Cactaceae) in vitro em resposta à substituição de nitrato de potássio (P.A.) por fertilizante comercial KNO_3

RESUMO: *Na micropropagação, o nitrato de potássio (KNO_3), reagente puro para análise (P.A.), utilizado no preparo dos meios de cultura, possui custo elevado e a sua aquisição depende de trâmites burocráticos, por se tratar de substância controlada pelo Exército Brasileiro. O objetivo deste trabalho foi avaliar o efeito da substituição do KNO_3 P.A. por fertilizante comercial (com fonte de KNO_3), encontrado livremente no comércio, na micropropagação de palma (Opuntia stricta (Haw.) Haw. cv Orelha de Elefante. Os tratamentos foram de seis concentrações do fertilizante (0; 0,5; 1; 1,5; 2 e 2,5 g L^{-1}) e um controle constituído de 1,9 g L^{-1} de reagente KNO_3, conforme mostrado nos sais MS. Avaliou-se a sobrevivência, tamanho e número de brotações do explante, e o valor da biomassa fresca. Após a aclimatização das mudas avaliou-se a sobrevivência, número de brotações, comprimento da parte aérea, número de raízes, formação da raquete, massa média da biomassa fresca, absorção de macronutrientes e alterações morfológicas das mudas. Os explantes inoculados em meio com fertilizantes nas concentrações de 0,0; 2,0 e 2,5 g L^{-1} não se desenvolveram. A resposta dos explantes nas concentrações de 0,5 e 1,5 g L^{-1} do fertilizante foram iguais aos desenvolvidos em meio contendo KNO_3, e na concentração de 1,0 g L^{-1}, em todas as variáveis, as médias foram superiores em relação as do controle. Dessa forma, constatou-se a viabilidade do uso do fertilizante no cultivo in vitro da palma, o que propiciou a eliminação dos entraves burocráticos e redução no custo de 99,12% na compra do produto.*
Palavras - chave: *macronutrientes, micropropagação, redução de custos.*

INTRODUCTION

The cultivation of cactaceae has grown due to its high foraging capabilities and ornamental characteristics, such as varied shapes and sizes. The prickly pear cactus cv. Elephant Ear (*Opuntia stricta* Haw.) stands out among these species due to its high productivity, low nutritional requirements, tolerance to drought and resistance to carmine mealybug (*Dactylopius opuntiae* Cockerel), the main cause

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of damages to cacti (SILVA et al. 2015). It is widely used in arid and semi-arid regions (ZINGALE, 2016), where forage cactus represents an important asset for animal feeding. However, conventional propagation methods are insufficient to meet the commercial demand for seedlings in these regions (BHAU & WAKHULU, 2015). Thus, micropropagation emerges as an alternative to obtain large-scale pest-resistant propagules.

In vitro cultivation enhances clonal propagation and accelerates genetic improvement of plant species, adding value to crops (GAVA & LOPES, 2012). However, micropropagation is expensive due to the material used in the preparation of the growing medium and the need for appropriate equipment and facilities to perform the technique (WEBER et al. 2015). In order to reduce costs, research has been conducted to find different sterilization protocols such as chemical sterilization (PAIS et al. 2016), the removal or reduction of inorganic salts (CHEE & POOL, 1987 and RIBEIRO & TEIXEIRA, 2008), and the replacement of inaccessible reagents with others, more affordable and freely traded.

Among the reagents that make up the growing medium, potassium nitrate (KNO_3), a high-purity macronutrient, is used at a great extent and, in addition to the high price, is an Army-controlled substance. This reagent is key in the induction and differentiation process of the aerial part of the plant, playing both a structural and enzymatic activation role, acting as an osmoregulator (MALAVOLTA et al. (1997) and TAIZ & ZEIGER, 2013). Replacing ACS KNO_3 with easy-to-purchase and lower-cost products tends to be a promising alternative to make micropropagated seedlings viable, provided their toxic effect on the explant is duly assessed (RIBEIRO et al. 2013).

To reduce costs in the acquisition of growing medium components and eliminating bureaucratic procedures for the purchase of reagents, this paper assessed the replacement of ACS KNO_3 for a commercial fertilizer and its effect on in vitro and exogenous development of the prickly pear cactus *Opuntia stricta* (Haw.) Haw.

MATERIALS AND METHODS

The vegetable matter from the prickly pear cactus used as an explant donor came from the inventory culture of the Embrapa Biotechnology Laboratory, Semi-arid, Petrolina, PE (09º04'16,4" S, 0 40º19'5,37" W). The plants were maintained for 120 days in a growth room with a 16-hour photoperiod, temperature of 25 ± 2 ºC and light intensity of 40 µmol m^2. The nutrient medium consisted of inorganic MS salts (MURASHIGE & SKOOG, 1962).

In vitro development was assessed at different concentrations of granular KNO_3, trade name Dripsolin (chemical composition (45% K_2O, 12% N and 1.2% S) in the growing medium, replacing the ACS reagent. We used the concentrations 0.5, 1.0; 1.5; 2.0 and 2.5 g L^{-1} of the fertilizer and for control purposes, we used ACS KNO_3 (1,9 g L^{-1}), as a reagent, in a total of seven treatments. The different concentrations were based on calculations of the chemical formulation in proportion to the ACS reagent.

The nutrient medium consisted of inorganic MS salts (MURASHIGE & SKOOG, 1962), and White vitamins (WHITE, 1943). The medium was supplemented with 0.1 g inositol, 30 g L^{-1} sucrose, 1.5 mg L^{-1} of 6 benzylaminopurine (BAP), 0.0625 mg L^{-1} naphthalene acetic acid (ANA) and 5 g L^{-1} agar as a gelling agent. The pH was adjusted to 5.9 ± 1 and the medium was sterilized by autoclaving (121 °C, 1.05 kg cm^2 for 20 minutes). After the preparation, 20 mL of the growing medium was distributed into containers glasses (50 mm x 200 mm).

Transverse segments of approximately 3 mm were placed in the growing medium and kept in the growth room at the Biotechnology Laboratory of the State University of Bahia (09º 25 '43.6" S, 40º 32' 14" W, 384 m), at a temperature of 27 ± 1 °C, 16-hour photoperiod and irradiance of 19 mol m^{-2} s^{-1}.

In vitro development was evaluated by counting the average number of shoots, the average number of shoots greater than or equal to two centimeters and the average value of fresh biomass. During the acclimatization, plants developed *in vitro* were transplanted to disposable cups (200 mL) filled with commercial substrate, identified according to each treatment and kept in a greenhouse with 75% shading. They were manually irrigated every four days. On the 35th day, plants were assessed with regard to the average number of shoots, average length of root and shoot, formation of cladode, average value of fresh biomass. In order to quantify the absorption of macronutrients according to the methodology proposed by Silva et al. (2009), we took a composite sample of each treatment. The seedlings were placed in five-liter plastic pots, filled with commercial substrate to allow the observation of morphological characteristics of the adult plant, and supposed changes due to the different dosages of potassium nitrate.

The experiment was conducted in a randomized design with seven treatments, five replications and three experimental plots. Count data

were into $\sqrt{x + 0.5}$ in order to meet the statistical assumptions, submitted to a variance analysis, and when proven significant ($P < 0.05$), the means were compared by the Tukey test at a 5% significance, using the Statistica software, version .8.0.

RESULTS AND DISCUSSION

Prickly pear cactus explants in a medium containing fertilizer at concentrations of 0.0; 2.0; and 2.5 g L^{-1} did not survive. Therefore, we were able to assess only plants obtained from treatments with concentrations of 0.5; 1.0 and 1.5 g L^{-1} of commercial fertilizer and compared to explants developed in a medium containing the ACS reagent (1.9 g L^{-1}), as a control treatment. Treatments with a growing medium containing 0.5 and 1.0 g L^{-1} of commercial fertilizer had a higher yield of shoots and shoots ≥ 2 cm than the control treatment (ACS $KNO_{3;}$). Regarding the average value of fresh biomass, the control treatment 1.0 g L^{-1} provided the highest value, while other treatments did not differ from the control treatment (Table 1).

In general, fertilizer dosages used in the preparation of the growing medium promoted responses on the explants of prickly pears cactus equal to or higher than those developed in the medium containing the ACS reagent. However, it should be noted that the 1.0 g L^{-1} fertilizer concentration resulted in higher values of all variables assessed. After 35th days of acclimatization, the plants showed the same responses in different concentrations of KNO_3 fertilizer in relation to the average length of the aerial part, average length of the root (MRL) and average formation of rackets / cladodes (ARI). As for the average number of sprouts (ANS) and average value of fresh biomass (FAVA), concentrations of 0.5 and 1.0 g L^{-1} produced higher values when compared to those obtained in treatments 1.9 (control treatment) and 1.0 g L^{-1} media (Table 2). During the acclimatization period, the plants adapted to the environment and showed a similar development; although, the seedlings were originated from micropropagation in growing mediums with different KNO_3 concentrations. The palm grew evenly and on day 20, the racket became expressive in the plants that went through the different treatments assessed, showing no morphological abnormality during its establishment. Leaf analysis allowed to ascertain macronutrient absorption, as a function of the different concentrations and source of KNO_3 (Table 3). The pace of nutritional absorption under the conditions proposed in the experiment was in the following ascending order: P <Mg <Ca <N <K.

Nitrogen is featured exclusively in the form of cation and anion (ammonium andnitrate, respectively) and is key in the performance of plant metabolic activities and absorption of other nutrients from the environment (REZENDE et al., 2008). It also directly influences the development of plants and, when in inappropriate quantities, may be highly harmful. (SASAMORI et al. 2016). Thus, the death of prickly pear explants, grown in a growing medium with 0; 2.0 and 2.5 g L^{-1}, may have been caused by the absence or excess of nitrogen in the form of nitrate, reported in the commercial fertilizer. However, fertilizer concentrations of 0.5 to 1.0 g L^{-1} added to the growing medium resulted in a good *in vitro* development of the prickly pear cactus, due to its low nutritional requirements, thus confirming the nutritional information of this species as assessed by LOPES et al. (2010), and this behavior is also observed in ex vitro growth. Thus, in order to reduce micropropagation costs, it is advised to use the smallest

Table 1 - Influence of different sources and potassium nitrate concentrations in the *in vitro* development of the prickly pear cactus *Opuntia stricta* (Haw.) Haw. cv. Elephant ear.

KNO_3 g L^{-1} Concentration	Surviving cultures%	ANS	ANS ≥ 2	AVFB
1.9 (MS)	100	2.05b	1.33b	1.56b
0.5	100	3.06a	2.31a	1.81b
1.0	100	3.04a	2.49a	3.91a
1.5	100	1.83b	1.37b	2.56b
CV		19.43	20.05	34.81

Averages followed by the same letter do not differ statistically from each other according to the Tukey test at a 5% probability; MS-salts (MURASHIGE & SKOOG, 1962); ANS - average number of shoots (cm); ANS ≥ 2 cm, average number of shoots greater than or equal to two cm; AVFB - average value of fresh biomass; CV - coefficient of variation.

Table 2 - Influence of different sources and potassium nitrate n the ex vitro development of the Prickly Pear *Opuntia stricta* (Haw.) Haw. cv. Elephant ear.

KNO_3	Surviving Cultures	ANS	CMPA	ARF	MRL	AVFB
gL^{-1}	%		cm	cm	cm	G
1.9 (MS)	100	1.84b	11.9a	2.43a	5.50a	15.96b
0.5	100	2.62a	10.44a	2.19a	6.50a	28.34a
1.0	100	2.41a	9.34a	1.97a	4.68a	25.89a
1.5	100	1.82b	9.60a	2.30a	5.27a	16.75b
CV		18,28	25,75	25,85	30,08	19,41

Averages followed by the same letter do not differ statistically from each other according to the Tukey test at a 5% probability; MS-salts (MURASHIGE & SKOOG,1962); ANS - average number of shoots (cm); ANS ≥ 2 cm - average number of shoots greater than or equal to two cm; CMPA - average shoot length (cm); ARF – average racket formation (cm); MRL – mean root length (cm); AVFB - average value of fresh biomass (g); CV - coefficient of variation.

amount of fertilizer, which meets the nutritional needs of the in vitro culture of the prickly pear without changing the physiological behavior under acclimatization and post-acclimatization conditions. The use of low concentrations does not change the macronutrient absorption process, such as nitrogen, phosphorus, potassium, calcium, magnesium, thus not interfering in the metabolic processes (TAIZ and ZEIGER, 2013). Considering the high proportion of KNO_3 in the ACS growing medium, the high cost and unavailability of nitrogen salts, there are some ongoing studies on alternative methods to make the micropropagation technique viable. KURITA & TAMAKI (2014) and SASAMORI et al. (2016), achieved a good development of bromeliads, when they reduced the amounts of salts and nitrogen compounds of the *in vitro* ACS medium. SOUSA et al. (2006) tested the reduction of ammonium nitrate and potassium nitrate in the micropropagation of coffee (*Coffea arabica* L. cv Rubi), and the best results were achieved in explants inoculated in a growing medium with 50% ammonium nitrate and 75% of potassium nitrate. Similar results were achieved by REZENDE et al. (2008), in the same coffee variety (Rubi), grown *in vitro*, in which the highest shoot growth occurred with the lowest concentrations of potassium nitrate and this development pattern was maintained in acclimatized plants. The result of reducing potassium nitrate concentration of the ACS medium in the in vitro culture of these crops is similar to the results reported for the prickly pear cactus. Under the conditions tested in this analysis, the lower KNO_3 concentrations (0.5 and 1.0 g L^{-1}), which corresponds to a 26.3 and 52.6% reduction, respectively, led to the highest growth under both *in vitro* and *ex vitro* conditions. Results showed that the reduction of potassium nitrate provides greater development, confirming that adjusting the salt concentrations of the ACS medium improves the quality of the resulting seedlings and also provides a cost reduction in the *in vitro* propagation. In addition to reducing inorganic salts, the replacement for more affordable products, eliminating bureaucratic and less

Table 3 - Influence of different sources and potassium nitrate concentrations on the nutritional absorption of *in* vitro micro propagated prickly pears *Opuntia stricta* (Haw.) Haw.

Concentrations	Ca^{+2}	Mg^{+2}	K +	P	NH^{+4}	NO^{-3}	Total N
KNO_3	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
1.9 (PA) ^{ns}	1.43	0.24	9.10	0.004	0.46	4.72	5.18
0.5 Fertilizer ^{ns}	1.32	0.24	10.10	0.002	0.58	4.83	5.41
1.0 Fertilizer ^{ns}	1.15	0.19	11.02	0.002	0.55	2.97	3.52
1.5 Fertilizer ^{ns}	1.11	0.16	10.01	0.002	0.51	2.83	3,34

The absorption rate was in an ascending order: P <Mg <Ca <N <K.
ns = The results did do not differ statistically.

expensive procedures, can be a promising alternative. VILLA et al. (2009), studied the possibility of inserting urea in the ratios of 0; 20; 40; 60; 80 and 100% in comparison to ACS ammonium nitrate in the micropropagation of the Blackberry (*Rubus rubus*) cv Tupy. In the absence of salt, plants yielded the highest amount of leaves, but plants developed in a medium with urea amounts higher than 20% presented reduced plant heights, number of leaves and fresh biomass, due to the resulting phytotoxicity. In the micropropagation of the pernambuco pineapple, urea was used at a concentration of 40%, replacing ACS ammonium nitrate, confirming the feasibility of the partial or total replacement of the agent, promoting a better plant development in a solid growing medium (MOREIRA et al., 2007). This response is associated with the nutritional specificity and phenological stage of each plant species in the absence of potassium nitrate. The prickly pear explants did not survive, as other plant species, such as the *Hypericum teretiusculum* A.St.Hil, wherein the reduction of nitrogenous compounds in the ACS growing medium harmed the development of plants (CAMPELO et al., 2020). RIBEIRO & TEIXEIRA (2008) tested the reduction and replacement of ACS KNO_3 for another fertilizer - potassium saltpeter – in the *in vitro* cultivation of Brazilian ginseng (*Pfaffia glomerata*), thus achieving a biomass increase. In this research, we obtained similar results in the in vitro and ex vitro cultivation of the prickly pear cactus, since its seedlings showed good development during and after acclimatization, maintaining a pattern and uniformity during growth. Regarding the nutrient absorption rate in the prickly pear cactus, SANTOS et al. (1990) reported an ascending order at the end of the production cycle: N <P <K <Ca. When propagated in a medium with different proportions of KNO_3 (ACS Reagent and granular fertilizer), the absorption rate was in an ascending order: P <Mg <Ca <N <K. Different absorption rates may be due to different physiological requirements throughout the development phases of the plant. Plant samples in this research were collected on the 35th day, under acclimatization conditions. Considering the development of the prickly pear cactus, under the conditions proposed for this paper, changing the ACS reagent for a commercial fertilizer is a promising alternative, since the acquiring ACS potassium nitrate (KNO_3) in Brazil is subject to the army's authorization (Ordinance N°. 118, 2019). Another feature that enhances the use commercial fertilizers as a viable alternative for potassium and nitrogen supply instead of the ACS reagent is the similar chemical composition (45% K_2O, 12% N and 1.2% S) and the ACS reagent (44% K_2O and 13% N). Therefore, replacing KNO_3 (ACS) minimizes the costs of setting a growing medium, as the current price of fertilizers is approximately R$ 170.20 each 25 Kg, while the ACS reagent costs R$ 780.00. The replacement resulted not only in a 99.12% cost reduction, but also eliminated procurement issues, as the purchase of fertilizer does not need to be authorized by the Armed Forces. When compared to the ACS medium protocol, a smaller proportion of commercial fertilizer (0.5 to 1.5 g L^{-1}) was enough to achieve the *in vitro* development and maintaining the ex vitro growth for the prickly pear cactus.

CONCLUSION

The 1.0 g L^{-1} concentration of a potassium nitrate-based fertilizer promoted the best growth response in the prickly pear cactus cv. Elephant Ear, both in and ex vitro, when compared to the ACS reagent. Replacing an ACS potassium nitrate reagent (KNO_3) for a similar commercial fertilizer is a promising alternative to the micropropagation of the prickly pear cactus cv. Elephant Ear, reducing costs of purchasing this reagent by 99.12% and eliminating paperwork.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the design and writing of the manuscript.

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