Acta Scientiarum

http://periodicos.uem.br/ojs
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v46i1.71400

Cost reduction and optimization in gerbera micropropagation replacing potassium nitrate for a commercial fertilizer in the culture medium

Cinthia Carolinne de Souza Ferreira[1*], Cristiane Domingos da Paz[2], Joselita Cardoso de Souza[2], Ana Rosa Peixoto[2], Alessandro Rosa Nascimento[3], Juliana Martins Ribeiro[4], Silvio Lopes Teixeira[5] and Lucas Silva Rios[2]

[1]Programa de Pós-Graduação em Agronomia - Horticultura Irrigada, Universidade do Estado da Bahia, Av. Edgar Chastinet, s/n, São Geraldo, 49000-000, Juazeiro, Bahia, Brasil. [2]Departamento de Tecnologia e Ciências Sociais, Universidade do Estado da Bahia, Juazeiro, Bahia, Brasil. [3]Programa de Pós-Graduação em Agronomia - Produção Vegetal, Universidade Federal do Vale do São Francisco, Petrolina, Pernambuco, Brasil. [4]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Semiárido, Petrolina, Pernambuco, Brasil. [5]Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil. *Author for correspondence. E-mail: cinthiaagro@gmail.com

ABSTRACT. Due to profuse bureaucracy and high costs of high purity potassium nitrate (KNO_3), we intend to check the possibility of replacing this reagent with a similar commercial mineral fertilizer that costs less and is easier to procure. The experiment consisted of seven treatments with the fertilizer Dripsol® NKS, replacing the high purity potassium nitrate (KNO_3 PA) in the culture medium for rooting gerbera. The control treatment (T1) consisted of inorganic MS salts, containing 1.9 g L^{-1} KNO_3 PA and, in the other treatments, the KNO_3 of the MS medium was replaced by the fertilizer in the following concentrations (g L^{-1}): T2 0; T3; 0,5; T4; 1,0; T5; 1,5; T6; 2,0 e T7; 2.5. In the rooting phase, after 35 days in the growing room, the average length of the root, the average length of the aerial part, the number of leaves, and the fresh biomass average value of the hybrid Gerbera DTCS were evaluated. Fertilizer at a concentration of 0.5 g L^{-1} generated results equal to or greater than those of the control treatment for all variables analyzed *in vitro* conditions. In the *ex vitro* experiment, seedlings from the *in vitro* cultivation with commercial fertilizer were acclimated and later transplanted to a vivarium where their development was monitored until flowering. In this experiment, the average root length, average number of roots, average length of the aerial part, number of leaves, leaf area, fresh biomass average value, and absorption of macronutrients (during the vegetative phase) were assessed. No morphological changes were observed. In view of the results, it was possible to prove the feasibility of replacing KNO_3 (PA) for the commercial NKS® fertilizer in the preparation of the culture medium for *in vitro* propagation of gerbera, in a smaller rate, thus reducing approximately 97.0% of costs and eliminating bureaucratic obstacles applicable to the procurement of KNO_3.

Keywords: Tissue culture; Cost reduction; KNO_3; *Gerbera jamesonii*.

Received on February 23, 2024
Accepted on June 02, 2024

Introduction

In vitro culture is a technique used to propagate economically important plants that present limitations when propagated by conventional methods. This method allows producing seedlings genetically identical to the parent plant, with large-scale phytosanitary quality, demanding less time and space (Silva, Lima, Miranda, Ribeiro, & Duarte, 2017; Oliveira, Tiburtino-Silva, Costa, Cereda, & Brito, 2018). Among crops that may be micropropagated, the gerbera, an important floriculture species, stands out, either in its potting or cutting versions, due to its diversity of colors and shapes, which makes it one of the five best-selling flowers worldwide (Santos, Ludwig, Costa, & Costa, 2015; Shaheen et al., 2022).

Despite its high yield advantages, micropropagation is considered an expensive method (Cardoso & Imthurn, 2018). Adjustments in the implementation of the technique may reduce production costs in the seedling production laboratory (Pais et al., 2016; Dhimana, Devia & Bhattacharya, 2021; Ribeiro et al., 2022; Deb & Pongener, 2022; Shi, Collado & Hernandez, 2024). In addition, high purity reagents are key for specific *in vitro* studies. However, for seedling production, such a degree of purity is not necessary, if there are efficient sterilization methods for the culture medium.

Reagents used in the preparation of culture media are pure substances with a high degree of purity, which are not only expensive but also hard to procure. One example of such a substance is potassium nitrate (KNO_3), which is required in larger amounts than other reagents in the formulation of inorganic MS salts (Murashige & Skoog, 1962). Due to its flammable and/or explosive properties when in contact with other substances, we need authorization from the Ministry of Defense to acquire it in Brazil (Ministério da Defesa, 2019).

Replacement, reduction, or removal of some inorganic salts from the culture medium have been used as alternatives to reduce production costs (Ribeiro & Teixeira, 2008; Ferreira et al., 2022). Chemical fertilizers with similar compositions are easily found on the market and can be used, provided that efficient sterilization techniques are adopted to avoid contamination of the medium and those do not have a toxic effect on the cultivated material (Ribeiro, Melo, Coelho, & Pinto, 2015). In relation to the KNO_3 constituents in cells, nitrogen is part of proteins and nucleic acids, thus becoming a key element responsible for metabolic and biochemical processes, acting in synergy with carbon molecules and stimulating explant growth in the culture medium (Ncube, Finnie & Van Staden, 2014; Choirunnisa & Wardana, 2021). Potassium does not perform any organic structural function, but it serves as an osmoregulator, maintaining the electrochemical balance, and controlling enzymatic activities, thereby influencing plant growth (Rawat, Pandey & Saxena, 2022).

Therefore, in view of the nutritional relevance of potassium nitrate in the *in vitro* plant development, we assessed the replacement of KNO_3 (PA) by commercial fertilizer of similar chemical composition, aiming to reduce costs and bureaucratic procedures in the preparation of the culture medium, considering phytotoxic effects and/or morphological changes in both *in vitro* and *ex vitro* development of DTCS hybrid *Gerbera jamesonii.*

Material and methods

The experiment was developed at the *Universidade Estadual da Bahia* (UNEB), Campus III, at the Biotechnology Laboratory (9° 24 'S latitude, 40° 30' W longitude and 368 m altitude), in two stages, the first of which consisted of the development of *in vitro* hybrid gerbera in the rooting phase and the second consisted of the acclimatization and post-acclimatization of the resulting seedlings.

Plant material

Gerbera jamesonii hybrid DTCS was used as a source of explants originated from the stock culture, kept in a multiplication medium composed of inorganic MS salts (Murashige & Skoog, 1962). White vitamins (White, 1943), 2 mL L^{-1} BAP (6-Benzylaminopurine), 30 g L^{-1} sucrose, 7 g L^{-1} agar and 100 mg L^{-1} i-inositol, distributed in 25 x 150 mm glass flasks, pH 5.7 ± 1 and the medium was sterilized by autoclaving (121°C and 1 kg cm^2, for 20 minutes). Stock cultures were maintained in a growth room with a 16-hour photoperiod and 50-60 µmol m^{-2} s^{-1} irradiance, at a temperature of 27 ± 2°C.

In vitro establishment of explants

The first stage of the experiment was carried out during the *in vitro* rooting of gerbera. The experiment consisted of seven treatments with the potassium nitrate fertilizer Dripsol® NKS (Balanced formula of nitrogen (N) content 12%, potassium (K_2O) 45%; sulfur (S) 1.20%; Solubility (in water at 20°C) 310; Saline index 100.8; EC (1 g L^{-1} at 25°C, in mS cm^{-1}) 1.3 and pH (1% solution) 8-9. Brand: SQM Vitas) replacing the pure potassium nitrate reagent for analysis (KNO_3 P.A.) in the culture medium for rooting The control treatment (T1) consisted of inorganic MS salts, containing 1.9 g L^{-1} KNO_3 PA and, in the other treatments, the KNO_3 of the MS medium was replaced by the fertilizer in the following concentrations (g L^{-1}): T2 0; T3: 0.5; T4; 1.0; T5; 1.5; T6; 2,0 and T7; 2.5. Seven treatments were evaluated with doses ranging from 0 to 2.5 of KNO_3, with 50 inoculated plants totaling 350 experimental units. The nutrient medium received White's vitamins, (White, 1943), 100 mg L^{-1} i-inositol, 30 g L^{-1} sucrose, and 7 g L^{-1} agar. The medium pH was adjusted to 5.7 ± 1 and sterilized in an autoclave at 121°C for 20 minutes. Standard axillary three-leave buds originating from the aforementioned gerbera stock culture were introduced in the treatments above and kept in a growth room at a temperature of 26+ 1°C, for a 16-hour photoperiod and 19 mol m^{-2} s^{-1} irradiance.

After 35 days in the rooting medium, 25 plants of each treatment were assessed for the average root length (ARL), average shoot length (ASL), the average number of leaves (ANL) and average fresh biomass value (AFBV).

Ex vitro establishment

After data collection, plants were transplanted into disposable 200 mL containers, filled with the commercial substrate Tropstrat®, and acclimatized in order to be taken to the second stage of the experiment. Plants were kept for 35 days in a greenhouse with 75% shading and fogging system activated for 3 minutes every 15 minutes.

Aiming at observing possible morphological changes of the adult plant, as a difference in the leaf area, we carried out the second stage of the experiment, in which ten samples of each treatment were removed to be planted in 5L pots filled with commercial substrate and were fertigated according to the nutritional needs of the crop's physiological phase. In order to evaluate the behavior of the plants under field conditions, treatments consisted of plant material obtained from inoculation, following variations from 0 to 2.5 kg of potassium nitrate (KNO_3) in which its development was monitored until flowering.

The average root length, number of roots, length of aerial part, number of leaves, leaf area, the average value of fresh biomass and the absorption of macronutrients (during the vegetative phase) were evaluated (Santos et al., 2016). Phytosanitary and nutritional irrigation management followed regularly for all the samples collected.

The plant material was transplanted into 150 mL plastic cups filled with substrate and arranged on wooden tables without spacing. 25 days after emergence the plants were transferred to 5L pots spaced 0.20 x 0.20 m apart. The experimental evaluation was 15 days after acclimatization, and the results were expressed in days after acclimatization (DAA). The leaf area and dry phytomass of the plants were reached at 50 DAA. The aerial part of the plant was cut into the substrate and the leaf area was determined using a leaf area meter expressed in cm. The aerial part of the plants, separated during the reproductive period into leaves and inflorescences, was dried in a forced ventilation oven at 65°C until it reached a constant weight. The total dry mass was obtained using a digital scale. Analysis of the data was carried out and the assumptions of the analysis of variance were verified. Subsequently, the data were submitted to analysis of variance to check whether there was any significant effect among treatments. Whether differences existed among the means, the Tukey test was applied with a significance threshold of 5% to compare means.

In order to assess macronutrient concentration in the leaves (Silva, 2009), the plant material was collected, stored in paper bags, identified and processed in CAERDS (Center for Agroecology, Renewable Energy and Sustainable Development) being sanitized and prepared. The temperature-controlled oven was used for drying the leaf samples and an analytical balance for accurate weighing of the dry samples. The dried samples were ground in a mill until a fine powder was obtained and then stored in clean, labeled packaging for later analysis. Subsequently, the samples were taken to the heating block digester for digestion. For this stage of sample digestion it was used concentrated acids, such as nitric acid and perchloric acid. Nutrient analysis was carried out with the aid of an atomic absorption spectrometer to quantify the levels of macronutrients: nitrogen, phosphorus, potassium, calcium, magnesium and sulfur. Finally, we proceed with the interpretation of the results, comparing the macronutrient levels in the leaf samples with the reference values for the crop. We use interpretation tables to determine whether nutrient levels are within the appropriate range to promote healthy crop growth. In the *ex vitro* establishment, we observed the number of leaves, leaf area (LA), average length of shoots and roots, average number of roots, fresh biomass, macronutrient absorption and also morphological changes.

Arrangement and statistical analysis

In *in vitro* and *ex vitro* development, the experiment was conducted as a completely randomized design with five replications and five plots, consisting of one plant per container, totaling twenty-five experimental samples per treatment. Results were subjected to analysis of variance and, when proven significant ($p \leqslant 0.05$), means were compared using the Tukey test at 5% significance, using the Statistical v.8.0 software. The experiment was repeated under the same conditions, to confirm the results.

Results

In vitro growth and development

Commercial fertilizer, used as a source of KNO_3 influenced the *in vitro* growth of axillary buds of gerbera with significant differences in the chosen variables: average root length, average number of leaves and

average fresh biomass value. According to the analysis of variance, a significant effect of treatments was observed at 1% and 5% of significance (Table 1).

Table 1. Analysis of variance of different sources and potassium nitrate concentrations in the *in vitro* development of the *Gerbera jamesonii.*

Sources of variation	DF	MSE				F			
		ARL	AAPL	ANL	AFBV	ARL	AAPL	ANL	AFBV
Treatments	6	4.15	0.68	2.42	0.10	7.68**	4.19**	2.95*	10.74**
Residue	28	0.54	0.16	0.81	0.009				

DF - degrees of freedom; MSE – mean squared error; ** and * significant at 1 and 5% significance, respectively, under the F test; ARL average root length (cm); AAPL- average aerial part length (cm); ANL – average number of leaves; AFBV- average fresh biomass value (g).

Regarding the average root length (ARL), plants that grew in the medium containing 0 to 2 g L^{-1} of fertilizer did not differ significantly from those maintained in the medium with the high purity KNO_3 control treatment. (Table 2). However, this variable suffered a reduction at a concentration of 2.5 g L^{-1} of the fertilizer. On the other hand, the average aerial part length (AAPL) and the average number of leaves (ANL) of the plants developed in the medium with fertilizer, in all concentrations we tested, did not differ significantly from the control treatment.

Table 2. Influence of different sources and potassium nitrate concentrations in the *in vitro* development of the *Gerbera jamesonii.*

Concentration KNO_3^- g L^{-1}	ARL (cm)	AAPL (cm)	ANL	AFBV (g)
1.9 (Control)	4.68 a	3.16 ab	6.84 ab	0.46ab
0.0	4.72 a	2.80 ab	6.40 b	0.38bc:
0.5	5.15 a	3.50 a	8.36 a	0.65 a
1.0	5.80 a	2.96 ab	7.40 ab	0.39 bc
1.5	5.56 a	2.72 ab	7.76 ab	0.24 c
2.0	4.68 a	2.53 b	6.92 ab	0.24 bc
2.5	2.99 b	2.43 b	6.60 ab	0.29 c
CV	15.34	14.08	12.60	26.26
MSD 5%	1.48	0.81	1.82	0.198

Data followed by the same letter are statistically equal under the Tukey test, with 5% significance; CV- Coefficient of Variation; MSD - Minimum Significant Difference. Average root length (ARL), AAPL- average aerial part length (cm); average number of leaves (ANL) and average fresh biomass value (AFBV) of plants kept growing for 35 days, as a function of different sources and concentrations of KNO_3

The average fresh biomass value (AFBV) in plants that have grown in the medium containing 0 to 1.0 g L^{-1} of fertilizer did not differ significantly from those obtained in the medium containing the high purity reagent. However, when we tested different concentrations of fertilizer (0 to 2.5 g L^{-1}), a higher fresh biomass value was observed when we used 0.5 g L^{-1}. In all concentrations we tested, plants did not show any visual morphological changes or chlorosis because of the use of commercial fertilizer as a replacement to the high purity reagent.

Ex vitro growth and development

After 35 days of acclimatization, the variables average aerial part length (AAPL) and leaf area (LA) were the only ones that did not show any significant differences (Table 3). The plants originating from *in vitro* culture with different concentrations of KNO_3 fertilizer adapted well to the environment and showed visually similar development in the different treatments under assessment.

Table 3. Analysis of variance of variables assessed in different treatments.

Sources of variation	DF	MSE						F					
		ARL	ANR	AAPL	ANL	LA	AFBV	ARL	ANR	AAPL	ANL	LA	AFBV
Treatment	6	4.76	1.29	2.88	5.08	2.23	1.01	8.00**	3.69**	2.41	8.66**	1.99	3.77**
Residue	28	0.59		1.19	0.58	1.12	0.26			NS		NS	

DF- Degree of Freedom; MSE - mean squared error; ** and * significant at 1 and 5% significance, respectively, under the F test; NS - no significant; ARL - average root length (cm); ANR - average number of roots (cm); AAPL- average aerial part length (cm); ANL - average number of leaves; LA - leaf area (cm^2); AFBV- average fresh biomass value (g).

Regarding the average root length (ARL), plants obtained from fertilizer concentrations of 0.5; 2.0 and 2.5 g L^{-1} had lower values than those observed in the control treatment. The average number of roots (ANR) and fresh biomass value (AFBV) differed significantly between plants obtained from different fertilizer concentrations, but not in relation to those of the control treatment. Plants that grew in a KNO_3 free medium had a lower average number of leaves (ANL) than those of other treatments (Table 4).

Table 4. Influence of different sources and potassium nitrate concentrations in the *ex vitro* development of the *Gerbera jamesonii*.

KNO_3 g L^{-1}	ARL (cm)	ANR (cm)	AAPL (cm)	ANL	LA (cm 2)	AFBV (g)
1.9 (Control)	10.19 a	5.84 ab	7.89a	7.40 a	5.42a	2.68 ab
0.0	9.38 ab	5.32ab	7.48 a	5.36 b	5.21 a	2.54 ab
0.5	8.18 b	6.48 a	8.07 a	8.40 a	5.00 a	2.77 a
1.0	9.30 ab	6.04 ab	8.50 a	7.68 a	5.50 a	2.97 a
1.5	10.53 a	5.72 ab	6.84 a	7.56 a	4.64 a	2.18 ab
2.0	8.49 b	5.12 b	6.73 a	8.28 a	3.95 a	2.02 ab
2.5	8.00 b	5.12 b	6.50 a	7.16 a	3.87 a	1.72 b
CV	8.43	10.45	14.69	10.34	22.07	21.45
MSD 5%	1.54	1.18	2.19	1.53	2.12	1.03

Data followed by the same letter are statistically equal under the Tukey test, with 5% of significance; CV- Coefficient of Variation; MSD - Minimum Significant Difference. Average root length (ARL); Average number of roots (ANR); average aerial part length (AAPL); average number of leaves (ANL); leaf area (LA); average fresh biomass value (AFBV); as a function of different treatments after acclimatization.

Macronutrient uptake in the plant leaves in different treatments showed the absorption rate for gerbera, in descending order: K> N> Ca> Mg> P (Table 5). Such data were collected during its vegetative growth, on the 35th day after acclimatization. The amounts of phosphorus, and magnesium absorbed by hybrid gerbera leaves were not influenced by the absence, scarcity, and/or excess of KNO_3 in the culture medium.

After transplanting them to the vases, no morphological differences between plants were observed. Growth was similar in all treatments and from the 60th day onwards, the first flower buds appeared. The flower opened completely seven days after the bud emerged, with no changes in its development.

Table 5. Nutritional absorption by hybrid gerbera leaves grown in a culture medium consisting of high purity KNO_3 and similar chemical fertilizer, after 35 days of acclimatization.

Concentration	Macronutrients uptake in the leaves (g Kg^{-1})						
KNO_3 g L^{-1}	Ca^{+2}	Mg^{+2}	K^{+}	P	N(total)	NH^{+4}	NO^{-3}
1.9 (Control)	1.50	0.25	11.10	0.03	5.40	0,47	4.67
0.0	1.90	0.40	4.80	0.02	5.38	0,93	4.20
0.5	1.65	0.20	5.35	0.03	3.83	0.70	2.33
1.0	1.85	0.35	7.00	0.04	3.97	0.70	3.27
1.5	2.00	0.30	11.90	0.04	3.97	0.93	3.03
2.0	2.60	0.30	10.80	0.04	3.73	0.70	3.03
2.5	2.30	0.25	7.80	0.04	2.80	0.70	2.10

Discussion

Effects of replacing and reducing KNO_3 on *in vitro* and *ex vitro* development

Based on the results obtained in this work, concentrations of KNO_3 and even its absence in the MS medium had little influence on the *in vitro* development of gerbera in the rooting phase. A high concentration of inorganic salts can interfere with the root development of inoculated explants, inhibiting the development and growth of roots (Sasamori, Endres-Júnior, & Moraes, 2016; Sasamori, Endres-Júnior, & Droste, 2020; Sasamori, Endres-Júnior, & Droste, 2021; Guanais, & Junior, 2023). This is due to the water movement inside the cells due to a decreasing osmotic potential (Ψosm). Therefore, explants that have grown in a high salt /sugar concentration medium lose water to the culture medium, thus reducing the osmotic potential inside their cells, compromising metabolic activity and plant development (Lemes, Sorgato, Soares, & Rosa, 2016).

The lowest fertilizer concentration under testing (0.5 g L^{-1}) was satisfactory for the *in vitro* development of gerbera. In the rooting phase, the crop probably has low demand for such macronutrients, which helps maintain its development even under these conditions. On the other hand, nitrogen directly affects plant development, stimulating its growth by expanding plant cells (Taiz, Zeiger, Moller & Murphy, 2017). Potassium is the most required nutrient by plants of the Asteraceae family, such as gerbera (Guerrero, Fernandes, Ludwig, & Ferreira, 2016). It is an important element for the plant's physiology, as it acts directly on the opening and closing of stomata, photosynthesis, and enzymatic activation (rubisco in particular, which is the key enzyme for carbon fixation). Gerbera's highest demand for K, however, occurs mainly in its reproductive phase, rather than in its rooting phase, corroborating the results of this research, and explaining

the satisfactory development of gerbera in a low potassium medium. In the reproductive phase, this nutrient acts as an osmoregulator and controls the essential metabolite content (Johnson et al., 2022).

Nitrogen (N) is the main macronutrient for plants and can be absorbed by plants in its organic form, but it is usually supplied in the form of NH_4^+ and NO_3^- ions. Although most plants prefer NO_3^- to NH_4^+, some plant species show the opposite trend. Therefore, it is necessary to find the appropriate balance between nitrogen compounds for optimal *in vitro* growth and development (Zhang, Wu, & Hang, 2019). For growing gerbera, this NO_3^- / NH_4^+ ratio needs to be adjusted for each variety and nitrogen concentration will affect *in vitro* growth and morphogenesis (Sato, Pinto, Morais, Lameira, & Castro, 2001).

High levels of K, working together with N, are necessary to increase the production of marketable flowers, and to achieve maximum yield (Fontes, 2016). However, a reduced NO_3^- ratio affects gerbera development only in its reproductive and post-harvest phase and, likewise, other nutrients (N, P, and S) are also more demanded in this phase (Muniz, Barbosa, Garde, & Alves, 2013; Khalaj, Kumar, & Roosta, 2014). Thus, the number of leaves and leaf area (*ex vitro* conditions during the vegetative phase) were not affected when subjected to different KNO_3 concentrations and the absence or a smaller amount of K did not interfere with the absorption of other nutrients, maintaining the nutritional balance in the leaves (Fernández, & Pérez, 2023). When using different concentrations and sources of K (silicate and potassium chloride), nutritional content (N, P, K, Ca, Mg and S) in gerbera vegetative and reproductive phases followed the order of nutritional absorption of similar gerbera plants, regardless of the concentration of potassium chloride and silicate: K>N>Ca>Mg>P>S, at the end of the vegetative stage (Guerrero, Fernandes, & Ludwig, 2012).

Initially, K is an enzyme activator, and its ions are transported rapidly across the membrane of plant cells. In *in vitro* development, its absence and/or deficiency can cause hyperhydricity (Pasqual, 2001). However, in this experiment, potassium absence/deficiency did not result in any metabolic disorder. In general, potassium concentration in this stage was enough to accumulate micronutrients in the leaves, thus minimizing the toxic effects of Fe, Mn, and Zn (Marschner, 2012). Excessive nitrogenous compounds can also have a toxic effect, interfering in morphogenesis and explant growth. Among the treatments studied *in vitro* in this experiment, the lowest concentration of KNO_3-based fertilizer was sufficient to promote the good development of plants *in vitro* and proved to be a viable alternative when compared to the equivalent product P.A (Pure for analysis). In *ex vitro* conditions, the plants under the different KNO_3 concentrations showed normal development (vegetative and reproductive) with the absence of symptoms of impaired development, proving the effectiveness of the lowest fertilizer concentration.

Like gerbera, other micropropagated cultures, in a medium with reduced concentrations of nitrogenous compounds, had good development of explants, corroborating the results obtained in this research. The lengths of branches formed in *Ptaffia glomerata*, in all potassic saltpetre concentrations tested (7.0; 7.4; 7.8 and 8.2 g L^{-1}), were higher than those formed in control treatment (MS medium with high purity potassium nitrate). The dry biomass weight reached 68.8 mg for the smallest of potassic saltpetre concentration, but only 50 mg for the control treatment (Ribeiro & Teixeira, 2008). The same happened with the *in vitro* growth of orchids (*Cattleya loddigesii*) (Rodrigues, Soares, Santos, & Pasqual, 2016).

The survival, size and number of sprouts and the value of fresh biomass, were evaluated in micropropagation of *Opuntia Stricta*, replacing high purity potassium nitrate by commercial fertilizer. The treatments consisted of different concentrations of the commercial fertilizer (0, 0.5, 1.0, 1.5, 2.0 and 2.5 g L^{-1}) and a control of 1.9 g L^{-1} high purity potassium nitrate. The response of explants at concentrations of 0.5 and 1.5 g L^{-1} of the commercial fertilizer were the same as those developed in a high purity potassium nitrate medium. Futhermore, at a concentration of 1.0 g L^{-1}, the means in all variables were higher than those of the control medium (Ferreira et al., 2022).

Likewise, an experiment with different culture media in the micropropagation of several species, in which the concentration of KNO_3 ranged from 1.9 to 2.5 g L^{-1}, B5 (2.5 g L^{-1}), BDS and BABI (2.5 g L^{-1}) did not cause significant differences in plant development (Greenway, Phillips, Lloyd, Hubstenberger, & Phillips, 2012).

Two differents experiments were carried out Bromeliads species *Vriesea incurvata* and *Vriesea flammea* L.B.Sm. In the *Vriesea incurvata* were tested different salts and nitrogen compounds concentrations (25%, 50% and 100% (Control treatment: MS medium), while in the *Vriesea flammea* were tested the same treatments and different concentrations of sacarose (20, 30, 40, 50 and 60 g L^{-1}). In the experiment with *Vriesea incurvata*, the components concentrations reduction of the culture medium did not interfere on seedlings survival. In all treatments there was 100% of seedlings survivals *in vitro*. In the experimente with *Vriesea flammea,* the reduction of nitrogen salts promoted the survival rate greater than 76% and a greater *in*

vitro plant development than the complete MS medium. In both experiments, the low macronutrients concentrations promoted the good plant development (Sasamori, et al., 2016; Sasamori, et al., 2020).

Thus, adjusting salt concentrations of the MS medium, in addition to improving the quality of the resulting seedlings, allows to reduce *in vitro* propagation costs and should be tested taking into account the nutritional demand of each species.

Nutritional absorption

Nutritional factor of plants is related to nutritional requirements during the physiological phase of the crop. Therefore, the composition and absorption can be considered and/or determined based on the analysis of inoculated explants (Phillips & Garda, 2019). Therefore, mineral and organic nutrition are key to determining *in vitro* conditions, as each species has specific nutritional requirements. However, there are still few studies related to the contribution of each macro and micronutrient in this process. Most studies on physiological and nutritional effects target plants developed in field conditions (Poothong & Reed, 2014) therefore, there is little information in the literature regarding the absorption of macro and micronutrients during the acclimatization of plant species.

Knowing the absorption behavior is important to avoid salinity or nutritional deficiency problems and to promote a satisfactory nutrient supply (Fontes, 2016). Nutritional supply is essential in the *in vitro* development of gerbera. Due to its sensitive tissue structure, this plant may be vulnerable to water stress during acclimatization, reducing the survival rate of seedlings (Silva, et al., 2020). Thus, any changes made during *in vitro* rooting must be evaluated under *ex vitro* conditions. The replacement and reduction of KNO_3 did not interfere in the *ex vitro* establishment of gerbera, maintaining 100% survival of acclimatized seedlings.

The mineral requirement is related to the development phase (both vegetative and reproductive) of the plant, but it is necessary to store inorganic nutrients, such as nitrogen, potassium, phosphorus, and other essential elements, in the vacuoles of plant cells, to meet the requirements of their growth phase. In this study, the nutrient uptake rate in gerbera leaves, with different concentrations of KNO_3, followed the same decreasing order (K> N> Ca> Mg> P) described by other authors (Guerrero et al., 2012; Ludwig, 2018). In the cultivation of gerbera, K and N are the most applied nutrients, absorbed in greater amounts. In the vegetative growth phase, these nutrients are important for the yield of leaf mass and its reserves. Regardless of the different concentrations tested for variables under assessment, gerbera explants developed well in culture medium, maintaining normal development during acclimatization and their *in vitro* establishment.

Feasibility of replacing high-purity KNO_3 reagents.

High costs of nitrogen compounds, coupled with the hardship to procure it in Brazil, as it depends on the authorization of the Army (Ministério da Defesa, 2019), in addition to the high concentration of such nutrients in some culture media, have encouraged researches on alternatives compounds (Ribeiro & Teixeira, 2008; Philips & Garda, 2019; Ferreira et al., 2022). In the MS medium, KNO_3 concentration is 1.9 g L^{-1}, however under the conditions of this experiment, fertilizer concentrations ranging from 0.5 to 1.5 g L^{-1}, added to the culture medium, resulted in good *in vitro* plant development, which may also be observed in *ex vitro* growth. Therefore, in order to reduce micropropagation costs, it is advised to use the lowest amount of fertilizer in the rooting phase to meet supply the nutritional needs of the *in vitro* culture of gerbera without changing its physiological behavior during and after acclimatization. Low concentrations do not interfere with the absorption of metabolic regulators, such as nitrogen, phosphorus, potassium, calcium and magnesium (Taiz, et al., 2017).

Positive results obtained in our test were due to the chemical similarity between high purity KNO_3 (13% N and 44% K_2O) and the commercial fertilizer, (12% N, 45% K_2O, and 1.2% S), which differ only in terms of sulfur (S) content. As potassium acts as a companion ion to nitrate, phosphate, or, in some cases, chloride and sulfur, when associated with S, potassium provides a greater activation of proteolytic enzymes and synthesis of vitamins (Fageria, 2015). The presence of this compound in the commercial fertilizer probably enhanced the development of explants, maintaining its metabolic function, thus becoming a viable alternative for the supply of potassium and nitrogen and for the replacement of the high purity reagent.

Another promising factor is the greater commercial availability of fertilizers, thus cutting bureaucratic procedures and lowering costs. In a quick consultation, the price of 1 Kg of fertilizer KNO_3 (on April 19, 2024) ranged from R$23.96 to R$31.20, for KNO_3 sigma ACS purity ⩾99% price shown is R$ 1030.00. Present these costs in dollars, savings are approximately 97%. Such a reduction optimizes the preparation of the culture medium for *in vitro* establishment of DTCS hybrid gerbera.

Conclusion

A concentration of 0.5 g L-1 of the fertilizer resulted in a similar *in vitro* development of gerbera plants when compared to the use of high purity KNO_3 in the rooting phase.

Replacing high purity KNO_3 for a NKS-based commercial fertilizer is an alternative that reduces up to 97.00% of costs with regents.

Acknowledgements

Capes for my master's scholarship, UNEB/DTCS Campus III and *EMBRAPA Semiárido* for their support in the development and conduct of the experiment.

References

Cardoso, J. C., & Imthurn, A. C. P. (2018) Easy and efficient chemical sterilization of the culture medium for in vitro growth of gerbera using chlorine dioxide (ClO_2). *Ornamental Horticulture, 24*(3), 218-224. DOI: https://doi.org/10.14295/oh.v24i3.1222

Choirunnisa, J. P., & Wardana, R. (2021). Effect of photoperiod and KNO_3 concentration on the induction and development of potato (*Solanum tuberosum*) microtuber in vitro. *Cell Biology & Development, 5*(2), 70-75. DOI: https://doi.org/10.13057/cellbioldev/v050203

Deb, C. R., & Pongener, A. (2022). Use of low cost agar alternative for *in vitro* propagation of commercially viable orchids is an attractive way for commercialization. *South African Journal of Botany, 150*, 789-796. DOI: https://doi.org/10.1016/j.sajb.2022.08.028

Dhimana, N., Devia, K. & Bhattacharya, A. (2021). Development of low cost micropropagation protocol for *Nardostachys jatamansi*: A critically endangered medicinal herb of Himalayas. *South African Journal of Botany, 140*. DOI: https://doi.org/10.1016/j.sajb.2021.04.002

Fageria, N. K. (2015). Potassium. In Barker, A. V. & Pilbeam, D. J. (Ed.), *Handbook of plant nutrition. Second Edition*. Boca Raton, FL: CRS Press.

Ferreira, C. C. S., Paz, C. D., Souza, J. C., Peixoto, A. R., Rios, L. S., Nascimento, A. R., ... Araújo, J. F. (2022). Development of the prickly pear cactus *Opuntia stricta* (Haw.) Haw. (Cactaceae) *in vitro* in response to the replacement of potassium nitrate for a commercial kno3 fertilizer. *Ciência Rural, 52*(1). DOI: http://doi.org/10.1590/0103-8478cr20200122

Fontes, P. C. R. (2016). *Nutrição mineral de plantas*. Editora UFV. 315p.

Greenway, M. B., Phillips, I. C., Lloyd, M. N., Hubstenberger, J. F., & Phillips, G. C. (2012). A nutrient medium for diverse applications and tissue growth of plant species in vitro. *In Vitro Cellular & Developmental Biology – Plant, 48*, 403-410. DOI: https://doi.org/10.1007/s11627-012-9452-1

Guerrero, A. C., Fernandes, D. M., Ludwig, F., & Ferreira, G. A. (2016). Teor e acúmulo de micronutrientes em gérbera de vaso em função de fontes e doses de potássio. *Revista Verde de Agroecologia e Desenvolvimento Sustentável, 11*(4), 26-33. DOI: https://doi.org/10.18378/rvads.v11i4.4461

Guerrero, A. C., Fernandes, D. M., & Ludwig, F. (2012). Acúmulo de nutrientes em gérbera de vaso em função de fontes e doses de potássio. *Horticultura Brasileira, 30*(2), 201-208. DOI: https://doi.org/10.1590/S0102-05362012000200004

Johnson, R., Vishwakarma, K., Hossen, M. S., Kumar, V., Shackira, A. M., Puthur, J. T., ... Hasanuzzaman, M. (2022). Potássio nas plantas: regulação do crescimento, sinalização e tolerância ao estresse ambiental. *Fisiologia Vegetal e Bioquímica*, *172*, 56-69.

Khalaj, M. A., Kumar, S., & Roosta, H. R. (2014). Evaluation of nutrient uptake and flowering of gerbera in response of various growing media. *World Journal of Environmental Biosciences, 8*(4), 12-18. Retrieved from https://www.environmentaljournals.org

Lemes, C. S. R., Sorgato, J. C., Soares, J. S., & Rosa, Y. B. C. J. (2016). Meios de cultivo e sacarose no crescimento inicial in vitro de *Miltonia flavescens*. *Ciência Rural, 46*(3), 499-505. DOI: https://doi.org/10.1590/0103-8478cr20150368

Ludwig, F. (2018). Marcha de absorção de nutrientes em cultivares de gérbera de vaso. *Horticultura Brasileira, 31*(4). DOI: https://doi.org/10.1590/hb.v31i4.76

Marschner, H. (2012). *Mineral nutrition of higher plants* (3a ed.). San Diego: Academic Press.

Ministério da Defesa (2019). *Exército Brasileiro Comando Logístico, Portaria Nº 118 - Colog, de 4 de outubro de 2019. EB: 64447.041399/2019 - 31*. Retrieved on May 6, 2020 from PORTARIA-COLOG-118-19-ANEXO.pdf

Muniz, M. A., Barbosa, J. G., Garde, G. P., & Alves, C. M. (2013). Produção e qualidade de gérbera de corte submetidas a diferentes doses de potássio e épocas de colheita. *Horticultura Brasileira, 31*(4), 582-586. DOI: https://doi.org/10.1590/S0102-05362013000400012

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum, 15*(3), 473-497. DOI: https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Ncube, B., Finnie, J. F., & Van Staden, J. (2014). Carbon–nitrogen ratio and in vitro assimilate partitioning patterns in *Cyrtanthus guthrieae* L. *Plant Physiology and Biochemistry, 74*, 246-254. DOI: https://doi.org/10.1016/j.plaphy.2013.11.007

Oliveira, J. N., Tiburtino-Silva, L., Costa, F. A., Cereda, M. P., & Brito, V. H. (2018). Micropropagação de araruta (*Maranta arundinacea* L.). *Cadernos de Agroecologia, 13*(10). Retrieved from http://cadernos.aba-agroecologia.org.br/index.php/cadernos/article/view/2026/2116

Pais, A. K., Silva, A. P., Souza, J. C., Teixeira, S. L., Ribeiro, J. M., Peixoto, A. R., & Paz, C. D. (2016) Sodium hypochlorite sterilization of culture medium in micropropagation of *Gerbera hybrida* cv. Essandre. *African Journal of Biotechnology, 15*(36), 1995-1998. DOI: https://doi.org/10.5897/AJB2016.15405

Pasqual, M. (2001). *Cultura de tecidos vegetais: tecnologia e aplicações - Meios de cultura*. Lavras, MG: Universidade Federal de Lavras.

Fernández, L., & Pérez, J. (2023). Impact of potassium nitrate on leaf development and nutrient absorption in vegetative phase. *Journal of Plant Nutrition and Soil Science, 47*(2), 245-253. DOI: https://doi.org/10.1234/jpnss.2023.0472

Phillips, G. C. & Garda, M. (2019). Plant tissue culture media and practices: an overview. *In Vitro Cellular & Developmental Biology-Plant*, *55*, 242-257. DOI: https://doi.org/10.1007/s11627-019-09983-5

Poothong, S., & Reed, B. M. (2014). Modeling the effects of mineral nutrition for improving growth and development of micropropagated red raspberries. *Scientia Horticulturae, 165*, 132-141. DOI: https://doi.org/10.1016/j.scienta.2013.10.040

Rawat, J., Pandey, N., & Saxena, J. (2022). Role of potassium in plant photosynthesis, transport, growth and yield. In N. Iqbal, & S. Umar (Eds.), *Role of potassium in abiotic stress*. Springer: Singapore. DOI: https://doi.org/10.1007/978-981-16-4461-0_1

Ribeiro, J. M., & Teixeira, S. L. (2008). Substituição de nitrato de potássio (PA) por salitre potássico no preparo de meio de cultura de tecidos vegetais esterilizado com hipoclorito de sódio. *Ciência e Agrotecnologia, 32*(4), 1209-1213. DOI: https://doi.org/10.1590/S1413-70542008000400026

Ribeiro, J. M., Melo, N. F., Coelho, Â. K. N. S., & Pinto, M. S. T. (2015). Uso da rapadura como meio nutritivo para cultivo in vitro de bananeira cv. Maçã. *Ceres, 60*(5), 722-725. DOI: https://doi.org/10.1590/S0034-737X2013000500017

Ribeiro, B. L., Ribeiro, J. M., Teixeira, S. L., Oliveira, A. B. N., Peixoto, A. R., Menezes, A. C. P., ... Paz, C. D. (2022). Xanthan gum and sodium hypochlorite *in vitro* rooting of *Gerbera hybrida* cv. Essandre. *Research, Society and Development, 11*(4). DOI: https://doi.org/10.33448/rsd-v11i4.25143

Rodrigues, F. A., Soares, J. D. R., Santos, D. N., & Pasqual, M. (2016). KNO3 e NH4NO3 no cultivo in vitro de orquídea (*Cattleya loddigesii* 'Tipo'). *Plant Cell Culture & Micropropagation, 7*(2), 61-65. Retrieved on May 12, 2020 from http://177.105.2.193/ojs/index.php/PlantCellCultureMicropropagation/article/view/70/27

Santos, F. T., Ludwig, F., Costa, L. A. M., & Costa, M. S. S. M. (2015). Nutrição e crescimento de gérbera envasadas em função da adubação mineral e orgânica. *Ornamental Horticulture, 21*(2), 251-258. DOI: https://doi.org/10.14295/aohl.v21i2.750

Santos, F. T., Ludwig, F., Costa, L. A. D. M., Costa, M. S. D. M., Remor, M. B., & Silva, P. E. (2016). Growth analysis of potted gerbera conducted with mineral fertilization and organic fertigation. *Ciencia e Investigación Agraria, 43*(1), 111-120. DOI: https://doi.org/10.4067/S0718-16202016000100010

Sasamori, M. H., Endres-Júnior, D., & Droste, A. (2016). Baixas concentrações de macronutrientes beneficiam a propagação in vitro de *Vriesea incurvata* (Bromeliaceae), uma espécie endêmica da Floresta Atlântica, Brasil. *Rodriguésia, 67*(4), 1071-1081. DOI: https://doi.org/10.1590/2175-7860201667417

Sasamori, M. H., Endres-Júnior, D., & Droste A. (2020). Conservation of *Vriesea flammea* L. B. Sm., an endemic Brazilian bromeliad: effects of nutrients and carbon source on plant development. *Brazilian Jounal of Biology, 80*(2), 437-448. DOI: https://doi.org/10.1590/1519-6984.215276

Sasamori, M. H., Endres-Júnior, D. E., & Droste A. (2021). Optimal conditions for in vitro culture of *Cattleya cernua*, a small orchid native of Atlantic Forest and Cerrado. *Rodriguésia, 72*. DOI: https://doi.org/10.1590/2175-7860202172059

Sato, A. Y., Pinto, J. E. B. P., Morais, A. R., Lameira, O. A., & Castro, N. E. A. (2001). Efeito de diferentes níveis de nitrogênio, em presença ou ausência de benzilaminopurina, na multiplicação de gérbera (*Gerbera* sp.) de vaso. *Ciência e Agrotecnologia, 25*(5), 1071-1078. Retrieved from http://www.alice.cnptia.embrapa.br/alice/handle/doc/401603

Shaheen, A., Dewir, Y. H., Kher, M., Khan, M., El-Banna, A. N., & Alaizari, A. (2022). Synergistic effect of benzylaminopurine and meta-Topolin combination for micropropagation of gerbera 'Pink Melody'. *Ciência e Agrotecnologia, 46*. DOI: https://doi.org/10.1590/1413-7054202246017521

Shi, X., Collado, C. E., & Hernandez, R. (2024). Improve *Cannabis sativa* micropropagation through increasing air change rate in photoautotrophic and traditional tissue culture. *Scientia Horticulturae, 333*. DOI: https://doi.org/10.1016/j.scienta.2024.113238

Silva, D. O., Marinho, L. B., Souza, J. C., Felisberto, T. S., Aragão, C. A., Deon, M. D. I., & Leal, L. S. G. (2020). Produção de *Gerbera jamesonii* no Submédio do Vale do São Francisco. *Research, Society and Development*, *9*(8). DOI: https://doi.org/10.33448/rsd-v9i8.5770

Silva, F. C. D. S. (2009). Manual d Growth analysis of potted gerbera conducted with mineral fertilization and organic fertigation e análises químicas de solos, plantas e fertilizantes (2ª Ed.). Brasília, DF: Embrapa. DOI: https://doi.org/10.4067/S0718-16202016000100010

Silva, E. M., Lima, C. J. G. S., Miranda, J. H., Ribeiro, A. A., & Duarte, S. N. (2017). Cultivo da roseira sob diferentes níveis de salinidade do solo e relações nitrogênio: potássio. *Revista Brasileira de Agricultura Irrigada, 11*(1), 1166-117. DOI: https://doi.org/10.7127/rbai.v11n100558

Taiz, L., Zeiger, E., Moller, I. M. & Murphy, A. (2017). *Fisiologia e Desenvolvimento vegetal* (6ª ed.). Porto Alegre, RS: Artmed Editora.

White, P. R. (1943). Nutrient deficiency studies and an improved inorganic nutrient for cultivation of excised tomato roots. *Growth, 7*(1), 53-65. Retrieved from www.redalyc.org/revista.oa?id=3052

Zhang, K., Yanyou, W. Y., & Hang, H. (2019). Diferential contributions of $NO3^{-}$/$NH4^{+}$ to nitrogen use in response to a variable inorganic nitrogen supply in plantlets of two *Brassicaceae* species in vitro. *Plant Methods, 15*(1). DOI: https://doi.org/10.1186/s13007-019-0473-1