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Micropropagation and genetic uniformity of *Kalanchoe daigremontiana* (Crassulaceae)[1]

Micropropagação e uniformidade genética de *Kalanchoe daigremontiana* (Crassulaceae)

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HIGHLIGHTS:

The different concentrations of sucrose had a similar influence on the growth of Kalanchoe daigremontiana *plantlets.*
The culture medium with 25% Murashige & Skoog (MS) salts also enhances the growth of K. daigremontiana *plantlets.*
K. daigremontiana *plantlets under sucrose concentrations, MS salts, and radiation maintain the same genetic profile.*

ABSTRACT: *Kalanchoe daigremontiana* is an ornamental species propagated exclusively asexually. However, no in vitro studies have been conducted to assess the relationship between changes in the culture environment and genetic variations of *K. daigremontiana*. Therefore, this study aimed to determine the optimal concentration of salts in the Murashige & Skoog (MS) medium and the optimal concentration of sucrose in the culture medium, in addition to the quality of light, in the development of in vitro plantlets from leaf explants of *K. daigremontiana*. Additionally, a genetic evaluation of the resulting plantlets was conducted to study phenotypic changes that could be attributed to somaclonal variation using Random Amplified Polymorphic DNA (RAPD) markers. For this purpose, leaf explants of *K. daigremontiana* were disinfected and inoculated in different salt concentrations (25, 50, and 100%) of MS medium without sucrose. The cultures were transferred to a growth chamber and subjected to three light conditions (white, blue, and red) with a 16-hour photoperiod, plus the absence of light, at a temperature of 27 ± 1 °C. Explants were also inoculated in 50% salt concentrations of MS medium supplemented with various concentrations of sucrose (control - without sucrose, 1.5, 3.0, 4.5, and 6.0%). *K. daigremontiana* leaf explants showed optimal development under white light and in any MS medium salt concentrations. A sucrose-free culture medium was viable, and no genetic variation was observed in the plantlets compared to the parent plants under the tested conditions.

Key words: in vitro propagation, ornamental plants, RAPD markers

RESUMO: *Kalanchoe daigremontiana* é uma espécie ornamental de propagação obrigatoriamente assexuada. Entretanto, nenhum estudo in vitro foi realizado para avaliar a relação entre mudanças no ambiente de cultivo e variações genéticas de *K. daigremontiana*. Portanto, o objetivo deste estudo foi estabelecer a melhor concentração de sais no meio Murashige & Skoog (MS) e a concentração de sacarose no meio de cultura, além da qualidade da luz, no desenvolvimento de plântulas de explantes foliares de *K. daigremontiana*. Além disso, realizamos uma avaliação genética das plântulas resultantes para estudar alterações fenotípicas que poderiam ser atribuídas à variação somaclonal utilizando marcadores da Amplificação Aleatória de DNA polimórfico (RAPD). Para tanto, explantes foliares de *K. daigremontiana* foram desinfestados e inoculados em diferentes concentrações de sais do meio MS (25, 50 ou 100%), sem sacarose. As culturas foram transferidas para sala de crescimento e submetidas a três condições de luz (branca, azul e vermelha) com fotoperíodo de 16 horas, mais a ausência de luz, sob temperatura de 27 ± 1 °C. Os explantes também foram inoculados em 25% das concentrações de sais do meio MS, suplementado com diferentes concentrações de sacarose (controle - sem sacarose; 1,5; 3,0; 4,5 e 6,0%). Os explantes foliares de *K. daigremontiana* desenvolveram-se melhor sob luz branca e em qualquer uma das concentrações de sal do meio MS. Meio de cultura isento de sacarose pode ser utilizado e nenhuma variação no perfil genético das plântulas foi observada em comparação com as plantas-mãe nas condições de cultura testadas.

Palavras-chave: propagação in vitro, plantas ornamentais, marcadores RAPD

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Introduction

Ornamental plants have been gaining an important space in the horticultural industry (Mubarok et al., 2023), not only for their ornamental value but other economic interests. In this context, *Kalanchoe daigremontiana*, besides being used as an ornamental species (Naz et al., 2009), has compounds as flavonoids and bufadienolides that can be used against tumors (Stefanowicz-Hajduk et al., 2020).

In plant physiology, this species is considered a model species for studies on the acid metabolism of Crassulaceae (CAM) (Garcês & Sinha, 2009), and recently novel genes that confer plant drought resistance were described for this species (Wang et al., 2018). Furthermore, *K. daigremontiana* is an attractive species for studying specific biological processes related to plant asexual reproduction (Winiarczyk et al., 2024).

Micropropagation is a powerful tool for propagating plants with economic interest, as this technique provides disease-free plants. Although *K. daigremontiana* is a plant species that reproduces asexually, in vitro propagation studies of this species allow for the development of ideal conditions for the multiplication of *Kalanchoe* plants, especially those of sterile species incapable of sexual reproduction (Winiarczyk et al., 2024). However, no in vitro studies have been conducted to assess the relationship between changes in the culture environment and genetic variations of *K. daigremontiana*.

Although *K. daigremontiana* can be micropropagated by leaf explants, few studies report the processes that involve micropropagation of this species (Kim et al., 2006; Garcês & Sinha, 2009; Naz et al., 2009; Winiarczyk et al., 2024). Several factors act in the plants' micropropagation, among them the culture medium, the irradiance conditions (Rojas-Vargas et al., 2023), and the carbon source (Zhou et al., 2024) highlight over the rest. Depending on the type of explant, morphogenetic route, and cultivation time, the exposure of plant cells to stressful conditions, such as chemical substances in the culture medium, for example, sugar, can hinder adequate tissue morphogenesis due to incorrect genetic expression (Ranghoo-Sanmukhiya, 2021), which can lead to somaclonal variants (Larkin & Scowcroft, 1981).

One of the biotechnological techniques used to identify this variation is the use of molecular markers that detect variation in the sequence of closely related genomes between the original plants, and the somaclones plants regenerated through tissue culture (Duta-Cornescu et al., 2023).

Taking into account the studies mentioned above, this study aimed to establish the best concentration of salts in the MS medium (Murashige & Skoog, 1962) and sucrose concentration in the culture medium, in addition to irradiance conditions, in the development of plantlets from in vitro leaf explants of *K. daigremontiana*. Furthermore, a genetic evaluation of the resulting plantlets was performed to study phenotypic changes that could be attributed to somaclonal variation using Random Amplified Polymorphic DNA (RAPD) markers.

Material and Methods

The experiment was conducted in the Laboratório de Biotecnologia Vegetal (LBV) of the Centro de Ciências Agrárias (CCA) of the Universidade Federal da Paraíba (UFPB), Areia - PB (6° 58' 10.9" S, 35° 42' 56.6" W, and an altitude of 600 m). *K. daigremontiana* leaf explants (Figure 1A) were used, obtained from adult plants grown on a substrate, and kept in pots in the greenhouse.

The leaf explants of *K. daigremontiana* were removed with tweezers, placed in Petri dishes (90 mm in diameter), taken to the laboratory, and washed under running water. In a laminar flow chamber, the explants were immersed in 70% (v/v) ethanol for one minute and then washed with distilled, deionized, and autoclaved water (DDA). Then, they were submerged in a 0.01% (v/v) mercury chloride ($HgCl_2$) solution for 10 minutes and rinsed three times in DDA water. Subsequently, they were submerged in 2% sodium hypochlorite for 10 minutes and rinsed thrice in DDA water.

Three distinctive experiments were conducted: Experiment I: Influence of different salt concentrations and light on in vitro plantlet growth; Experiment II: Influence of different sucrose concentrations on the *in vitro* plantlet growth; and Experiment III: Analysis of genetic fidelity in *K. daigremontiana* plantlets developed *in vitro*.

In Experiment I, disinfected leaf explants (5 mm^2) of *K. daigremontiana* were inoculated in test tubes (25 × 150 mm) containing 15 mL of different concentrations of salts of the MS medium (25, 50, or 100% - MS/4, MS/2, or MS, respectively), solidified with 0.7% (w/v) agar (Sigma®) and without sucrose. The other components of the MS culture medium were kept the same as the composition of the original MS medium. The pH of the medium was adjusted to 5.7 ± 0.1 before the inclusion of the agar (Sigma®) and autoclaved at 120 °C and for 15 min. One explant was inoculated per tube, and the incubation conditions in the growth chamber were as follows: four irradiance conditions with the fluorescent lamps: white (350-700 nm; 20 µmol m^{-2} s^{-1}), blue (430-490 nm; 17 µmol m^{-2} s^{-1}), and red (630-700 nm; 12 µmol m^{-2} s^{-1}), with a photoperiod of 16 hours, and absence of light, and all explants were kept at a constant temperature of 27 ± 1 °C.

The experimental design was completely randomized and arranged in a 3 × 4 factorial scheme (culture medium salt concentrations × irradiance conditions), totaling 12 treatments, with 10 replicates each, resulting in 120 plantlets.

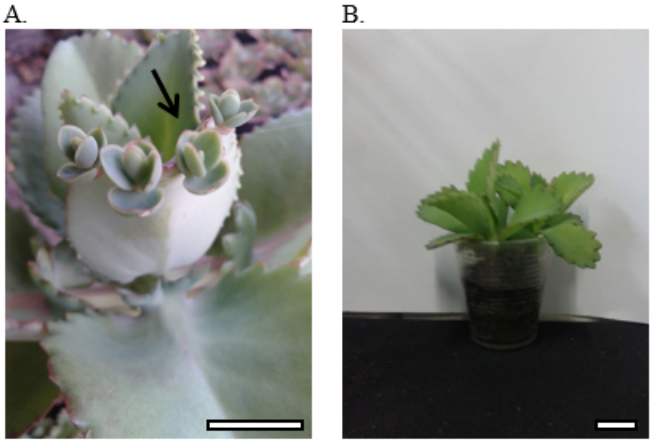


Figure 1. *Kalanchoe daigremontiana* leaf explants (arrow), bar = 1 cm (A). Plantlets derived from *K. daigremontiana* leaf explants in the acclimatization process, bar = 4.3 cm (B)

After 30 days from the beginning of the experiments, the plantlet length, number of leaves, leaf length, number of roots, root length, number of shoots, shoot length, and fresh matter weight were evaluated. A portable chlorophyll meter SPAD-502® (Minolta Camera Co., Ltd., 1989) was used to estimate the green color intensity.

In Experiment II, the disinfected explants were inoculated in test tubes (25 × 150 mm) containing 15 mL of 50% of the concentrations of MS salts culture medium with different concentrations of sucrose: control (without sucrose), 1.5, 3.0, 4.5, and 6.0% (m/v). The medium was solidified with 0.7% (m/v) agar (Sigma®). The other components of the MS culture medium were kept the same as the composition of the original MS medium. The pH of the medium was adjusted to 5.7 ± 0.1 before the inclusion of the agar (Sigma®) and autoclaved at 120 °C and for 15 min. One explant (as obtained in Experiment I) was inoculated per tube. All cultures were kept in a growth chamber under a 16-hour photoperiod provided by white light (fluorescent light) with an irradiance of 20 μmol m^{-2} s^{-1} at 27 ± 1 °C.

The acclimatization of the plantlets (Figure 1B) in both experiments was conducted, as reported by Santos et al. (2021).

The statistical design was completely randomized, totaling five treatments, with 10 repetitions each, totaling 50 analyzed plantlets. After 30 days from the beginning of the experiments, the plantlet length, number of leaves, leaf length, number of roots, root length, number of shoots, shoot length, and fresh matter weight were evaluated. The estimate of green color intensity was conducted as indicated in Experiment I.

Experiment III analyzed the genetic fidelity in *K. daigremontiana* plantlets developed in vitro. The extraction of genomic DNA was performed using the methodology described by Doyle & Doyle (1990). For that, samples of 200 mg of young leaf tissue of *K. daigremontiana* collected in bulk from ten replicates of each treatment from Experiment I and Experiment II and from the parent plant that originated the explants were subjected to DNA extraction.

The DNA amplification reactions were performed using a final volume of 25 μL, composed of 23 μL Master Mix (Techne TC-Plus Bibby Scientific Ltda®) (buffer 1X + 3 mM $MgCl_2$ + 200 mM dNTP + 1 mM primer + 1 U Taq DNA polymerase) and 2 μL of genomic DNA (50 ng) of the sample. A blank sample to check contamination was made. No contamination was identified. The amplifications were performed in a thermocycler (Techne TC-Plus Bibby Scientific Ltda®), with initial denaturation of 94 °C for 5 min and 30 cycles, being: 94 °C for five minutes, 94 °C for one minute, 36 °C for one minute and 72 °C for two minutes) and, finally, the last cycle at 72 °C for five minutes. After amplification, the samples were stored at -20 °C until used. Eleven primers (UB-03, UB-04, UB-05, UB-06, UB-07, UB-08, UB-09, UB- 10, UB-11, UB-12, UB-14) (Operon Technologies Inc., Alameda, CA, EUA) were used to obtain the RAPD markers. For the electrophoresis gel (1.5%), it was used TAE 1X. Furthermore, a 10 μL aliquot of the products amplified for each sample in the RAPD reaction was used, which were transferred to Eppendorf tubes, and 2 μL of loading buffer (4 g of glucose were added + 0.025 g of bromophenol blue + 0.025 g of xylene cyanol + 10 μL of distilled water), in each well. Then, 10 μL of the sample was applied to each well of the 1.5% agarose gel, TAE 1X buffer, 1.08 g of agarose, 0.06 μL ethidium bromide (10 mg mL^{-1}). DNA Ladder - 1kb (Invitrogen™) was used as a DNA molecular weight marker. The electrophoresis was run at 70 volts for approximately two hours. The gel was visualized in a T26M UV 302 nm transilluminator, and the image was captured in an EasyDoc 200 Photo Documentation system. After gel band readings, a binary matrix was built, with (0) indicating the absence and (1) the presence of bands and the number of amplified bands for each primer.

The obtained data from Experiments I and II were submitted for analysis of variance, and the means were compared using the Scott-Knott test at $p \leq 0.05$. Measurements on the effect of different concentrations of sucrose on the plantlet length, root length, and the SPAD index were subjected to regression analysis. In addition, the models were adjusted by the significance of the F test at $p \leq 0.05$. All statistical analyses were performed using the software Genes (Cruz, 2013). For Experiment III, the percentage of the amplification was calculated.

Results and Discussion

The interaction between irradiance conditions and culture medium salt concentrations only affected the number of shoots (NS). The irradiance conditions significantly influenced all variables except for shoot length. The culture medium did not influence the evaluated traits (Table 1).

There was an increase in plantlet length, leaf length, and number of shoots when plantlets were grown under white and red lights. Considering the number of leaves, the number of roots, root length, and fresh matter weight, the white light showed the highest means (Figure 2 and Table 2). The lowest means for all evaluated traits, except fresh matter weight, were observed under blue light and the absence of light (Table 2). Similar to our results, in the literature, some authors reported that white and red lights were beneficial in micropropagation (Ushijima et al., 2017; Park et al., 2018). These irradiance

Table 1. Summary of the analysis of variance for the effect of concentrations of MS (Murashige & Skoog, 1962) salts (CM) and different irradiance conditions (IC) in the morphological characteristics of *Kalanchoe daigremontiana* plantlets

Treatments	DF	p-value								
		PL	NL	LL	NR	RL	NS	SL	FMW	SPAD
IC	3	0.01**	0.01**	0.01**	0.01**	0.01**	0.05*	0.63ns	0.01**	0.01**
CM	2	0.13ns	1.23ns	0.06ns	0.07ns	0.06ns	0.54ns	0.06ns	0.06ns	2.19ns
IC × CM	6	0.29ns	1.99ns	0.08ns	0.68ns	0.07ns	0.05*	0.25ns	0.07ns	0.56ns
CV (%)		36.09	44.54	10.94	37.66	18.49	40.90	33.17	8.27	39.01

*; ** Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively; ns Not significant; DF - Degrees of freedom; CV – Coefficient of variation; PL - Plantlet length (cm); NL - Number of leaves; LL - Leaf length (cm); NR - Number of roots; RL - Root length (cm); NS - Number of shoots; SL - Shoot length (cm); FMW - Fresh matter weight (g); and SPAD - Chlorophyll index

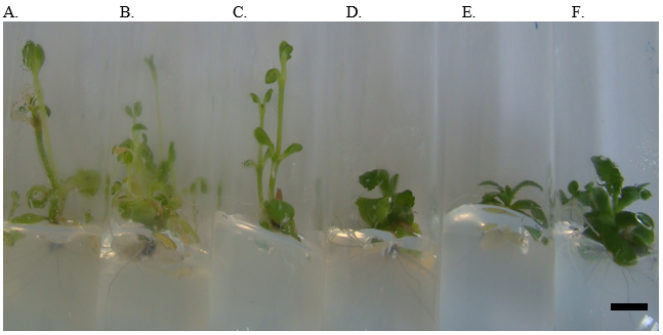


Figure 2. *Kalanchoe daigremontiana* plantlets inoculated under different irradiance conditions and concentrations of MS (Murashige & Skoog, 1962) salts: (A, B, and C) Red light - (25, 50, or 100% - MS/4, MS/2, or MS, respectively), respectively; and (D, E, and F) White light - MS/2, MS/4, and MS, respectively, bar = 1 cm

conditions directly influence the activation of phytochrome, promoting stem elongation and plantlet development (Ushijima et al., 2017; Park et al., 2018; Sarikhani & Sarikhani-Khorami et al., 2021).

The increase in the number of leaves and root length observed in white light was extremely important for plantlet development because, in the first case, an increase in the photosynthetic area provided by the increase in the number of leaves can result in a more efficient photoautotrophy as suggested by Victório et al. (2015). The increase in plant organs during in vitro cultivation under different irradiance conditions has also been reported in the literature. Pasa et al. (2012) reported that the increase in root length in white light permitted a greater absorption of water and nutrients, which enabled a greater growth of explants and, consequently, better adaptation of plantlets during the acclimatization process. According to Pedroso et al. (2017), the red light promotes a reduction in leaf area and root length due to the increase in the elongation of the internodes in response to the intensity of the far-red light and the use of endogenous auxins more intensively. Contrary to our results, the red light was better regarding the multiplication coefficient in *Stevia rebaudiana* (30% increase) than white light (Shulgina et al., 2021), indicating that each species has a different behavior under in vitro growth conditions.

The number of shoots in the MS/4 culture medium was higher when the plants grew under white and red light (Table 3). In contrast, in the MS/2 culture medium, only white light significantly affected the number of shoots, while red light did not differ statistically from blue light and the absence of light (Table 3). When red light was used, the number of shoots was greater than blue light and the treatment without light (dark) (Table 3).

There was a difference in the number of shoots for the culture medium when the plantlets were subjected to white light (Table 3). In MS/4 medium, the number of shoots was higher when the plantlets were subjected to red or white light, and for MS/2 and MS medium only in the white light (Table 3). Unlike our results for blue light, Rojas-Vargas et al. (2023) reported that a significantly higher percentage of shoots was obtained in explants of *Pinus ponderosa* developed under blue LEDs (light-emitting diodes) when compared to those exposed to red LEDs.

In Experiment II, it was observed that the sucrose concentrations did not influence plantlets' responses concerning variables except for plantlet length, root length, and SPAD index (Table 4, Figures 3 and 4). Furthermore, the explants were responsive in the sucrose-free culture medium (Figure 3).

The positive response of leaf explants of *K. daigremontiana* in the sucrose-free culture medium in the present study was very important, as it could be a key factor at the time of acclimatization because it allows an increase in the photosynthetic capacity of the plants. However, this result was possible in some species with natural light or with the

Table 3. Effect of different irradiance conditions and culture medium on the number of shoots in *Kalanchoe daigremontiana* plantlets

Irradiance conditions	Culture medium		
	MS/4	MS/2	MS
White	1.53 bA	1.87 bA	2.35 aA
Blue	0.90 aB	0.87 aB	1.04 aC
Red	1.69 aA	1.24 aB	1.62 aB
Dark	0.97 aB	1.16 aB	0.90 aC

Means followed by the same lowercase letters in the row or uppercase letters in the column constitute a statistically homogeneous group by the Scott-Knott at $p \leq 0.05$; different concentrations of salts of the MS (Murashige & Skoog, 1962) medium (25, 50 or 100% - MS/4, MS/2 or MS, respectively)

Table 2. Effect of the different irradiance conditions on the morphological characteristics of *Kalanchoe daigremontiana* plantlets

Irradiance conditions	PL	NL	LL	NR	RL	NS	FMW	SPAD
White	1.94 a	4.09 a	1.08 a	4.15 a	1.55 a	1.92 a	1.06 a	5.03 a
Blue	1.01 b	1.14 c	0.89 b	1.07 c	0.91 c	0.94 b	0.88 b	0.97 c
Red	1.83 a	2.67 b	1.04 a	2.48 b	1.24 b	1.52 a	0.93 b	2.72 b
Dark	1.22 b	1.28 c	0.87 c	1.28 c	0.92 c	1.01 b	0.88 b	0.87 c

Means followed by the same lowercase letters in the column constitute a statistically homogeneous group by the Scott-Knott test at $p \leq 0.05$; PL - Plantlet length (cm); NL - Number of leaves; LL - Leaf length (cm); NR - Number of roots; RL - Root length (cm); NS - Number of shoots; FMW - Fresh matter weight (g); and SPAD - Chlorophyll index

Table 4. Summary of the analysis of variance for the effect of different concentrations of sucrose (SC) in the morphological characteristics of *Kalanchoe daigremontiana* plantlets

Treatments	DF	Morphological characteristics								
		p-value								
		PL	NL	LL	NR	RL	NS	SL	FMW	SPAD
SC	4	0.01**	0.06 ns	0.06ns	0.06ns	0.05*	0.30ns	0.36ns	0.40ns	0.05*
CV (%)		30.70	40.59	27.69	38.75	39.03	51.38	52.13	46.80	54.58

*; ** Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively; nsNot significant; DF - Degrees of freedom; CV – Coefficient of variation; PL - Plantlet length (cm); NL - Number of leaves; LL - Leaf length (cm); NR - Number of roots; RL - Root length (cm); NS - Number of shoots; SL - Shoot length (cm); FMW - Fresh matter weight (g); and SPAD - Chlorophyll index

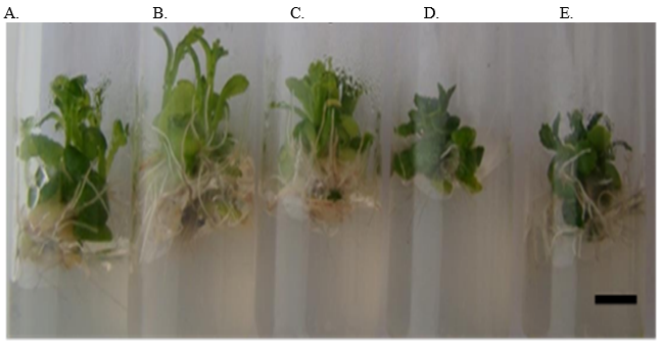
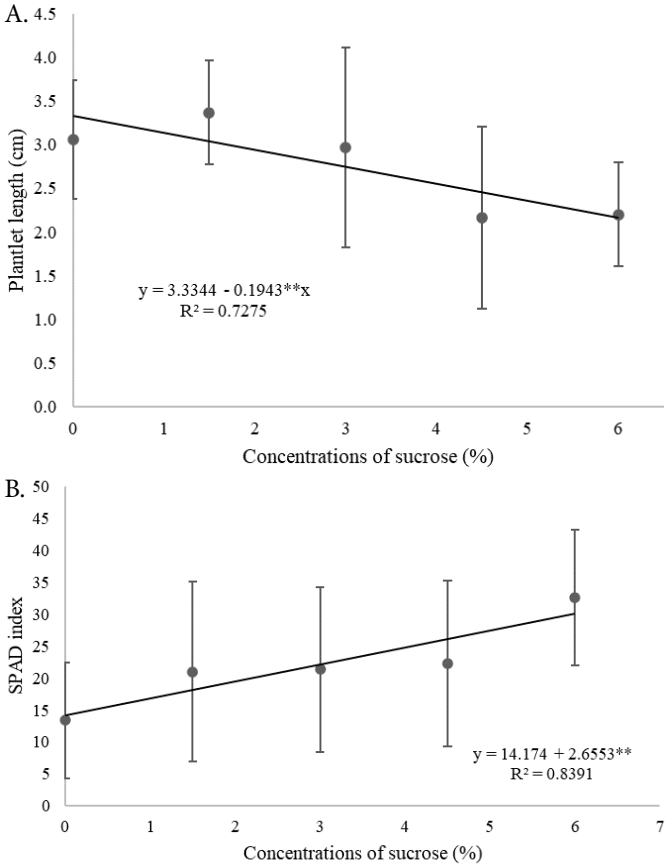


Figure 3. *Kalanchoe daigremontiana* plantlets inoculated under different concentrations of sucrose: (A) Control - without sucrose; (B) 1.5%; (C) 3,0%; (D) 4.5%; and (E) 6.0%, bar = 1 cm

supplementation of CO_2 in the cultivation environment. Santos et al. (2020) reported that fully photoautotrophic micropropagation was possible only under natural light in *Physalis angulata*, while Gago et al. (2021) only achieved this result when apical and basal sections of *Salix viminalis* were cultivated in bioreactors under high light intensity (150 µmol $m^{-2} s^{-1}$) and ventilated six times a day with CO_2 enrichment.

Our results showed that the increase of sucrose concentrations provoked a decrease in the plantlet length (Figure 4A) and root length (means: 1.82, 2.36, 1.57, 1.50, and 1.37 cm for the control - without sucrose, and for sucrose concentrations of 1.5, 3.0, 4.5, and 6.0%, respectively; y = 2.0748 – 0.1177*x; R^2 = 0.5063, * Significant at p ≤ 0.05 by the t-test) while the SPAD index increased with the sucrose concentration (Figure 4B). Plantlets can increase the SPAD index as an acclimation response to stress, as described by Tang et al. (2018), who observed a reduction in the size of potato leaves and an increase in chlorophyll content in leaves of plants under heat stress. Our results suggest that higher concentrations of sucrose may have caused water stress in the plantlets that led to increased chlorophyll content, but more studies must be conducted to confirm these results. Furthermore, the negative influence of 60 g L^{-1} of sucrose in the length of the plantlet and the root indicated that high concentrations of sucrose retard the in vitro growth of explants by reducing the availability of water resulting in stress in these explants. Contrary to our results, Ng et al. (2020) reported that 3.0% of sucrose promoted an increase in the elongation of the stem and the number of roots of *Hylocereus polyrhizus* in comparison to other concentrations of sucrose (control - without sucrose, 1.0, and 2.0%). However, endogenous sugars (glucose) levels in plant cells serve as signals for SnRK1TOR and allow growth and development to be maintained in vitro (Lucho et al., 2023).



** Significant at p ≤ 0.01 by the t-test. Vertical bars indicate standard deviation

Figure 4. Plantlet length (cm) (A) and the chlorophyll (SPAD) index (B) in plantlets of *Kalanchoe daigremontiana* cultivated on MS culture medium supplemented with different concentrations of sucrose after 30 days

The *K. daigremontiana* species reproduces asexually, and all somatic embryos are clones of the parent plant (Garcês et al., 2007). Based on that, it was expected to have no genetic variation, although depending on factors such as plant species, genotype, ploidy level, phytohormones, growth medium composition, and total culture time (Ranghoo-Sanmukhiya, 2021), tissue culture can cause somaclonal variation and directly cause genetic variation in plantlets obtained by this technique (Larkin & Scowcroft, 1981).

In Experiment III, it was observed that among the 11 primers tested, 92% presented amplifications. These primers generated 112 well-defined bands with an average of 10.18 bands per primer (Table 5).

When the plantlets of *K. daigremontiana* were maintained under different in vitro culture conditions, the plantlets were phenotypically different in terms of the number of roots and number of leaves (light and carbon source) (Figure 5). However, based on the evaluated RAPD electrophoretic profile, all bands were monomorphic for the loci accessed, showing that there was no somaclonal variation detected among plantlets of *K. daigremontiana* obtained by in vitro culture when compared to

Table 5. RAPD markers, sequence of nucleotide bases, and the total number of amplified bands in plantlets of *Kalanchoe daigremontiana*

Primer	Base sequence (5' - 3')	Number of amplified bands
UB- 03	GGGCGACTAC	14
UB- 04	GTGCGCAATG	11
UB- 05	TCGCATCCAG	10
UB- 06	CAGAAGCGGA	13
UB- 07	CACAGCGACA	13
UB- 08	CAAAGCGCTC	10
UB- 09	TCCCCATCAC	2
UB- 10	TGCGGGTCCT	12
UB- 11	CAGGATTCCC	10
UB- 12	GTGGAGTCAG	10
UB- 14	CAGCACTGAC	7
Mean		10.18
Total		112

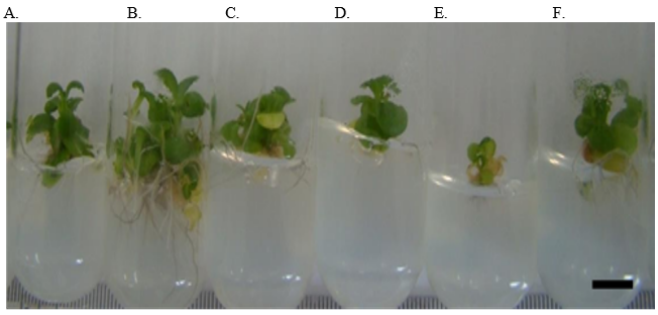
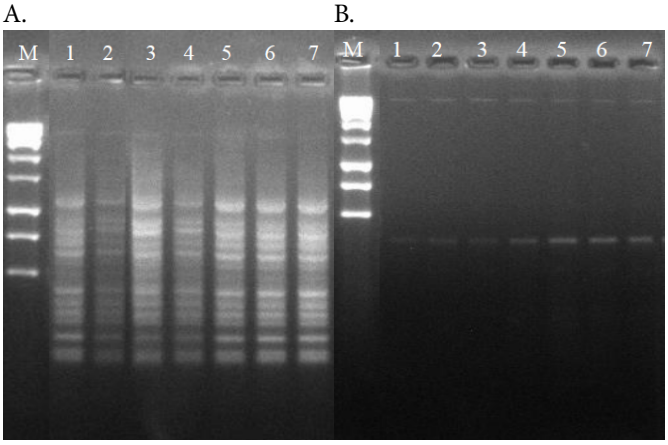


Figure 5. *Kalanchoe daigremontiana* plantlets grown up under different in vitro culture conditions: A, B, and C refer to red, white, and blue light, respectively; and D, E, and F refer to control (without sucrose), 3.0, and 6.0% of sucrose, respectively, bar = 1 cm

the profile of the matrix plants based on the tested primers (Figure 6). Therefore, the variation found was only in the phenotypic features caused by the different treatments of the in vitro culture. Based on this, we conclude that the monomorphic bands reported in this study reproduce the genetic fidelity existing among *K. daigremontiana* plantlets, showing the protocol's success and thus indicating the possibility of large-scale production of clones through micropropagation of this species.



1 - Parent plant; 2 to 7 - Plantlets developed in vitro under different growing conditions - 2, 3, 4 - red, white, and blue light, respectively; 5, 6, 7 - Control (without sucrose); 3.0, and 6, 0% sucrose concentrations, M - Molecular marker

Figure 6. Electrophoretic profile in 1% agarose gel, from RAPD amplification tests performed with genomic DNA samples from seven plantlets of *Kalanchoe daigremontiana*, using primers UB-03 (A) and UB-09 (B)

Conclusions

1. Leaf explants of *Kalanchoe daigremontiana* developed best under white light and at any of the concentrations of salts in the MS medium, including 25% of the concentrations of salts in the MS medium.

2. The leaf explants of *K. daigremontiana* developed in all concentrations of sucrose, and a sucrose-free culture medium can be used for the micropropagation of this species.

3. Under the cultivation conditions, no somaclonal variation was detected based on the electrophoretic profile obtained with the 12 RAPD primers.

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