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RESEARCH PAPER

Impact of colchicine on leaf morphology and stomatics of *Kalanchoe tubiflora* (Harv.) Raym.-Hamet (Crassulaceae)

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ABSTRACT

The demand of medicinal plants for consumption is greatly increasing worldwide. The conventional breeding programs are generally dependent on the environment prone to biotic and abiotic stresses. These added to the low content of secondary metabolites at harvest, bring the need for artificial development of polyploid individuals as an alternative to increase productivity. Consequently, the present study evaluated the effect of different colchicine concentrations and exposure time, on *Kalanchoe tubiflora* leaf morphology and stomata. Initially, *K. tubiflora* seedlings were harvested and submitted to colchicine concentrations of 0, 0.025, 0.05 and 0.1 % and at two exposure times (24 and 48 hours). Subsequently, morphological measurements such as plant height, leaf width, leaves number, leaf length, leaf thickness and leaf volume every 15 days were made for 16 weeks after planting. Then, the stomata were characterized, taking into account the width, length, stomatal index and the number of chloroplasts per stoma. A significant increase in leaf morphology was found in colchicine treatments of 0.025 % at 48 h and 0.1 % at 24 h. A significant increase in stomatal morphology with the treatment of 0.025 % at 24 h was also recorded. This shows that the correct application of colchicine in term of quantity and time could produce greater growth in a short period and increase the biomass of *K. tubiflora* medicinal plant.

Keywords: chloroplasts, mitotic inhibitor, medicinal plant, ploidy

РЕЗЮМЕ

Салазар С.А.М., Квинтеро И.Д.К., Бастос В.Д.У. Влияние колхицина на морфологию листьев и устьицы *Kalanchoe tubiflora* (Harv.) Raym.-Hamet (Crassulaceae). Спрос на лекарственные растения значительно возрастает во всем мире. Традиционные программы разведения, как правило, зависят от окружающей среды, а также от биотических и абиотических стрессов. Это обуславливает необходимость искусственного получения полиплоидных особей в качестве альтернативы для повышения продуктивности. В настоящем исследовании оценивалось влияние различных концентраций колхицина и времени его воздействия на морфологию листьев и устьиц *Kalanchoe tubiflora*. Первоначально его сеянцы были подвергнуты воздействию колхицина в концентрациях 0, 0,025, 0,05 и 0,1 % при двух выдержках (24 и 48 часов). Впоследствии в течение 16 недель после посадки каждые 15 дней производились морфологические измерения таких параметров как высота растения, ширина листа, количество листьев, длина листа, толщина листа и объем листа. Затем для устьиц были определены ширина, длина, устьичный индекс и количество хлоропластов на стому. Значительное увеличение размеров листьев было обнаружено при обработке колхицином на 0,025 % при экспонировании в течение 48 ч и на 0,1 % – 24 ч. Также было зарегистрировано значительное увеличение параметров устьиц при обработке на 0,025 % в течение 24 часов. Это показывает, что правильное по концентрации и времени применение колхицина может привести к большему росту за короткий период и увеличению биомассы лекарственного растения *K. tubiflora*.

Ключевые слова: хлоропласты, ингибитор митоза, лекарственное растение, плоидность

Переведено редколлегией

The genus *Kalanchoe* belongs to the Crassulaceae family which includes about 125 described species (Salazar et al. 2018). Fifty six species are native to Southern and Eastern Africa while the remaining 60 species are native to Madagascar (Pokhrel & Karsai 2015, Vlielander et al. 2018). It is currently distributed throughout the world especially in hot climates (Mora & Hernández 2016, Richwagen et al. 2019). They mostly include Crassulacean Acid Metabolic plants (Gotoh et al. 2019), and they are commercially produced throughout the year (Huang & Chu 2012). Despite its often exotic appearance, *Kalanchoe* has ethnobotanical uses, it is called "miraculous leaf" for its use in the treatment of various ailments (Milad et al. 2014, Richwagen et al. 2019).

Many species from the genus are used in natural and pharmaceutical medicine since ancient times, due to they produce secondary metabolites rich in alkaloids, triterpenes,

glycosides, flavonoids, steroids (bufadienolides) and lipids, which help to treat health problems such as bronchitis, mosquito bites, respiratory infections, tuberculosis, epilepsy, varicella, heart clinical treatments, among others (Kolodziejczyk et al. 2017, Agarwal & Shanmugam 2019). Its effect on prostate cancer has been proven (Arias-González et al. 2018), and they have been object of bioengineering with the development of a type of transgenic *Kalanchoe pinnata* (Lam.) Pers. which produces an antimicrobial peptide (Zakharchenko et al. 2016, Lebedeva et al. 2017). The quality of kalanchoe ornamental plants has become increasingly important (Mibus et al. 2014) with a production of more than 150 million of *Kalanchoe blossfeldiana* Poelln. plants per year in Europe. In 2012, *Kalanchoe* placed second on the list of the 25 best-selling indoor plants in the world's largest flower auction in the Netherlands, with a sales volume of USD 55 million and 77 million units sold (Mibus et al. 2014). The genus has also contributed to plant breeding, as it is the case of the isolation of resistance genes to *Phytophthora nicotianae* (Oh & Son 2008).

Kalanchoe tubiflora (Harv.) Raym.-Hamet is a succulent plant from Madagascar also known as *Bryophyllum* and *Kitchingia* (Chernetskyy 2012, Huang et al. 2013). It is characterized by its high level of cardiac glycosides. For this reason, it has gained importance in the pharmaceutical and medicinal fields. In addition, it is a very popular ornamental plant (Kulus 2015). *K. tubiflora* has great capacity to prevent the loss of water, being able to generate seedlings even under conditions of extreme drought (Luo et al. 2015). It has also shown antitumor activity caused by bufadienolides (Hsieh et al. 2012, Huang et al. 2013, Solis et al. 2018). Huang et al. (2021) demonstrated the autophagy induction in highly metastatic human lungs CL1-5 cancer cells, by three *K. tubiflora* isolated bufadienolides. Likewise, the findings made by Hsieh et al. (2016) showed that *K. tubiflora* caused senescence of mice cells (A549) xenografted with human tumor cells. It is also used to treat wounds, allergies and skin diseases (Hsieh et al. 2012). This omni-potential characteristic has caused an increase in demand of the species. Therefore, the development of efficient multiplication protocols is justified (Kulus 2015). It is necessary to develop a cultivation system or methodology that guarantees a sustainable production of secondary metabolites rich in alkaloids, triterpenes, glycosides, flavonoids, steroids (bufadienolides) and lipids (Arias et al. 2009), developing alternatives to obtain higher production volumes, through genetic selection and breeding programs (Sattler et al. 2016, Quintero et al. 2009).

In this order of ideas, the use of colchicine has been successful, demonstrating greater growth and increase in different plants' biomass production (Urwin 2014) since it allows an increase of its vegetative parts caused by polyploidy (Sadat et al. 2017, Matos 2014). Colchicine is the most commonly used mutagen in plants due to its efficiency and reliability in the induction of polyploidization (Ade & Rai 2010, Salma et al. 2017). It is also an effective mitosis inhibiting agent which destabilizes and depolymerizes microtubules during the formation of mitotic spindle (Lu et al. 2012). This will prevent pairs of chromosomes from splitting and moving towards the opposite pole during anaphase, thereby cause two sets of chromosomes to remain in a single nucleus that forms polyploid cells (Ade & Rai 2010, Eng & Ho 2019).

The most important advantage of polyploidy is that plants tend to have better performance and morphological characteristics, such as height and size of the plant organs (Hannweg et al. 2016), and the increase in biomass in general (Urwin 2014). To develop an efficient polyploidization protocol for a species, updated research is essential. It is estimated that only 15 % of angiosperm species were formed as a result of polyploidization (Wood et al. 2009). This indicates that the formation of a new polyploid is rare (Eng & Ho 2019). To idicate polyploid plants, indirect methods are performed as morphological characters (Sánchez & Matos 2012), cytological methods measuring the stomatal size and density, the number of chloroplasts in stomata guard cells (Sadat et al. 2017, Orrillo & Bonierbale 2009, Choque et al. 2007, Standring et al. 1990) and by chromosome counting (Matos 2014, Salazar et al. 2018). For the foregoing, taking into account the medicinal and ornamental importance, the following study evaluates the effect of different concentrations and exposure time of colchicine on foliar morphology and *K. tubiflora* stomata.

MATERIAL AND METHODS

Cultivation conditions and plant material

This study was carried out at the biology laboratory of Francisco de Paula Santander University located at 7°54'01.1"N and 72°29'15.6"W, in Cúcuta city, Colombia, under climatic conditions of the area, characterized by being a tropical dry forest at 320 m a.s.l., average temperature of 27.2°C, air relative humidity oscillates between 62 % and 79 % during the year, being higher in the months of November and December and lower towards the middle of the year and an annual rainfall average of 622 mm/year (IDEAM). *K. tubiflora* seedlings were collected from a mother plant collected in Cúcuta municipality, Colombia. 80 seedlings were taken, with 1 cm height average and a developed root system (Salazar et al. 2018). Subsequently, the seedlings were submitted to different colchicine concentrations (0.025, 0.05 and 0.1 % w/v) and exposure times (24 and 48 h) in total darkness, taking the distilled water as control (Table 1). Next, they were planted in 1-litre polyethylene bags on a substrate composed of vermiculite, rice husk and clayey soil in proportions 1:1:1.

Table 1. *Kalanchoe tubiflora* treatments with different colchicine concentrations and exposure times.

Treatments	Colchicine concentrations	Exposure times (hours)
T1	0.1	24
T2	0.05	24
T3	0.025 %	24
T4	Control (H_2O)	24
T5	0.1 %	48
T6	0.05	48
T7	0.025 %	48
T8	Control (H_2O)	48

Morphological analysis

For the morphology analysis, aspects such as plant height (*HP*), foliar length (*FL*), leaves number (*LN*), leaf width (*LW*), foliar thickness (*FT*), number of seedlings were considered (*NS*) and foliar volume (*FV*), using the formula:

$$V = \pi \times LH \times AH \times EH / 12,$$

according to the methodology used by Salazar at al. (2018). The previous variables were taken with a millimeter rule with the exception of the FV, where a caliper gauge was used. The measurements were made every 15 days, for 16 weeks after sowing.

Stomatal analysis

For stomatal characteristics such as width, length and chloroplast number, the methodology developed by Salazar et al. (2018), where transparent adhesive tape was used to remove the epidermis from the abaxial zone, from the central part of the sheet of approximately 1 cm^2. The tape was fixed on a slide, then the specimen was placed under the LEICA compound microscope model DME 500. It was then observed at 100X objective. This was repeated 10 times per sheet. The obtained photos were analyzed with the INFINITY ANALYZE 6.5 software.

To determine the stomatal index (*SI*), the number of stomata and epidermal cells per field of observation was counted at 400X magnification. The *SI* was calculated using the formula implemented by Wilkinson (1979):

$$SI = NS / (CE + NS) \times 100$$

Where *NS* is the number of stomata per visual range and *CE* number of epidermal cells in the visual range. 10 replications were made per treatment.

Experimental design and statistical analysis

We processed the data using Microsoft Office Excel and Statistica, version 13.3 (StatSoft Inc., USA). The results are represented as the means ± standard errors of means. Analysis of variances (one-way ANOVA) using Fisher's protected least significant difference (PLSD) post-hoc test was applied. A difference of $P < 0.05$ was considered significant.

RESULTS

Morphological study

According to the morphological characteristics evaluated in *K. tubiflora*, variations were found in the applied treatments (Table 2, Fig. 1). Regarding the plant height (PH) assessment, statistically significant differences were observed in the treatments with colchicine at 0.1 % for 24 h (T1) and the treatment with 0.025 % during 48 h (T7) causing a greater increase with respect to the rest of treatments (20.5 cm). As a result of the leaves number (*LN*), it is highlighted that the treatments T1 and T7 did not have significant differences among themselves, with 30 and 31 respectively and being the highest values in this variable. A marked trend was observed in the T1 and T7 treatments (Table 2 and Fig. 2), these being the highest values obtained in the foliar length (*FL*), with 10 cm and 11 cm respectively, without significant differences between them (Table 2). On the other hand, a greater average value of 3 cm is observed in the treatment T1 in the leaf width (*LW*). Then it is observed that the treatments T1 and T7 maintain the trend with higher values for foliar thickness (0.35 and 0.32 cm respectively). Regarding the foliar volume (*FV*) it is observed that T1 produced a value of 2.7 above the rest of the treatments, it is also observed that the lowest value (0.15) occurred in T8. Finally, the production of shoots is highlighted only by treatments T1, T6 and T7 (Table 2) from week 10.

Stomatal analysis

In the stomata analysis, it can be observed that T3 was the highest number (37.78 µm) in the stomatal length, without significant differences with the treatments T1, T2,

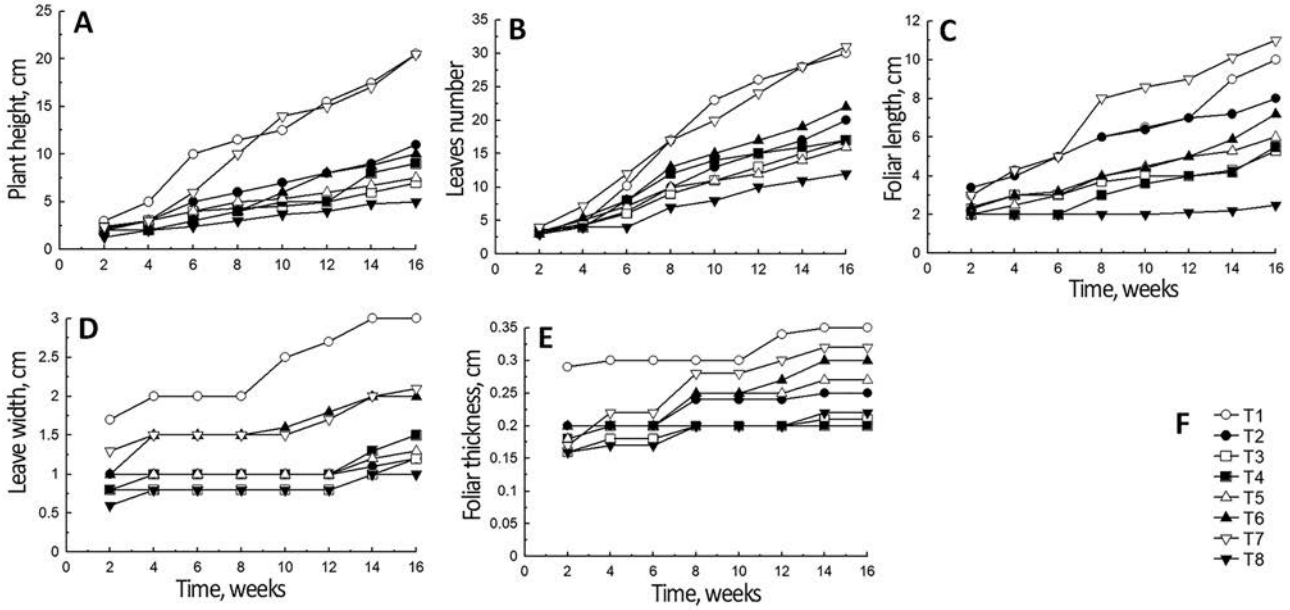

Figure 1 *Kalanchoe tubiflora* (Harv.) Raym.-Hamet morphological study (A) *PH*: plant height; (B) *LN*: leaves number; (C) *FL*: foliar length; (D) *WL*: leave width; (E) *FT*: foliar thickness. (F) treatments symbology

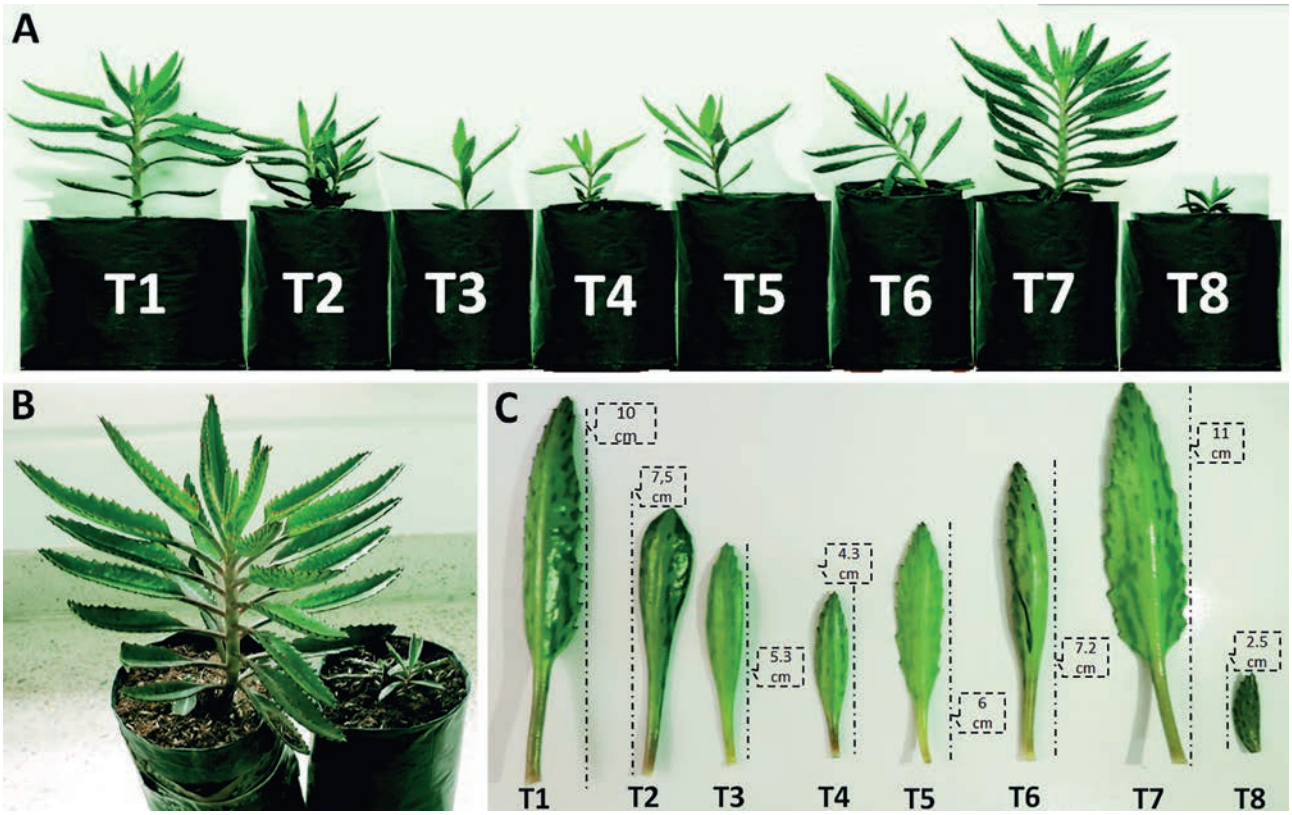

Figure 2 Colchicine effect on *Kalanchoe tubiflora* (Harv.) Raym.-Hamet leaf morphology: A – comparison of treatments in week 16; B – comparison of treatments T7 and T8; C – *K. tubiflora* leaves at 16 weeks

T5, T6 and T7, while T8 presented the lowest value of 28.66 µm, without significant differences with T4 (Table 3 and Fig. 3). Regarding stomatal width, it is observed that T3 has the highest value at 23.01 µm with significant differences to the rest of the treatments and T8 presents the lowest value at 17.5 µm without significant differences with T4 (Table 3). Similarly, we can see a trend with T3 treatment, which is the one with the highest number of chloroplasts per stoma (28.8) without significant differences with T7 (28.6), it should be noted that T4 had the lowest chloroplasts per stoma rate with 18.0 (Fig. 3D), without significant differences with T8 (Table 3, Fig. 3). In addition, T1 presented the highest stomatal index with a value of 29.36 (Table 3, Fig. 4A), followed by that presented by T7 with 28.6. In the case of T4, it showed the lowest average value with 10.3 with significant differences to the other treatments (Table 3, Fig. 4).

DISCUSSION

Morphological study

It is observed that the treatments T1 (0.1 % for 24 h) and T7 (0.025 % for 48 h) presented statistical homogeneity in *PH*, *LN*, *FL* and *FT*. As well as a production of 6 and 4 seedlings, respectively, it is therefore noteworthy that the use of the T7 treatment reduces around 75 % the amount of colchicine and therefore reduces the procedure cost compared to T1. Likewise, it can be seen that T1, is similar to what Sánchez & Matos (2012) and Matos (2014) observed in their studies, where the use of colchicine at 0.10 % in *Aloe vera* L. for 48 hours caused significant increases in plant height, foliar length and foliar volume. In the same way, concordance with what was found by Sadat et al. (2017), where the treatment with 0.1 % during 6 h caused a higher plant height in *Trachyspermum ammi* Sprague. On the other hand,

Table 2. Colchicine effects on *Kalanchoe tubiflora* foliar morphology.

Treatments	Colchicine effects on *K. tubiflora* foliar morphology						
	PH **(cm)**	***LN*** **(cm)**	***FL*** **(cm)**	***WL*** **(cm)**	***FT*** **(cm)**	***FV***	***NS***
T1	20.5±0.9^{a}	30±2.5^{a}	10±2.5^{a,b}	3.0±0.14^{a}	0.35±0.03^{a}	2.7^{a}	6
T2	11±0.7^{b}	20±0.7^{b,c}	8±0.7^{b,c}	1.2±0.14^{d}	0.25±0.02^{b,c}	0.62±^{c,d}	0
T3	7±1.5^{c,d}	17±1.4^{c,d}	5.3±0.4^{c}	1.2±0.3^{c,d}	0.21±0.03^{c}	0.33±^{d}	0
T4	9±0.9^{b,c}	17±1.4^{c}	5.5±0.3^{c}	1.5±0.07^{c,d}	0.2±0.01^{c}	0.43±^{d}	0
T5	7.5±1.1^{c}	16±1.4^{d}	6±0.7^{c}	1.3±0.3^{d}	0.27±0.01^{b,c}	0.55±^{d}	0
T6	10±1.5^{b}	22±2.9^{b}	7.2±1.7^{c}	2.0±0.2^{b}	0.3±0.06^{a,b}	1.13±^{c}	2
T7	20.51.1±^{a}	31±2.2^{a}	11±1.8^{a}	2.1±0.3^{b}	0.32±0.05^{a,b}	1.90±^{b}	4
T8	5±0.7^{d}	12±0.7^{e}	2.5±2.8^{d}	2.5±0.3^{a,b}	0.22±0.008^{c}	0.15±^{d}	0

The means ± SD values with different letter of each column show statistically significant differences, according to Tukey´s test ($P \leq 0.05$). *PH*: plant height; *LN*: leaves number; *FL*: foliar length; *WL*: leave width; *FT*: foliar thickness; *FV*: foliar volume; *NS*: number of seedlings. Evaluation carried out at 16 weeks after sowing. SD = standard deviation.

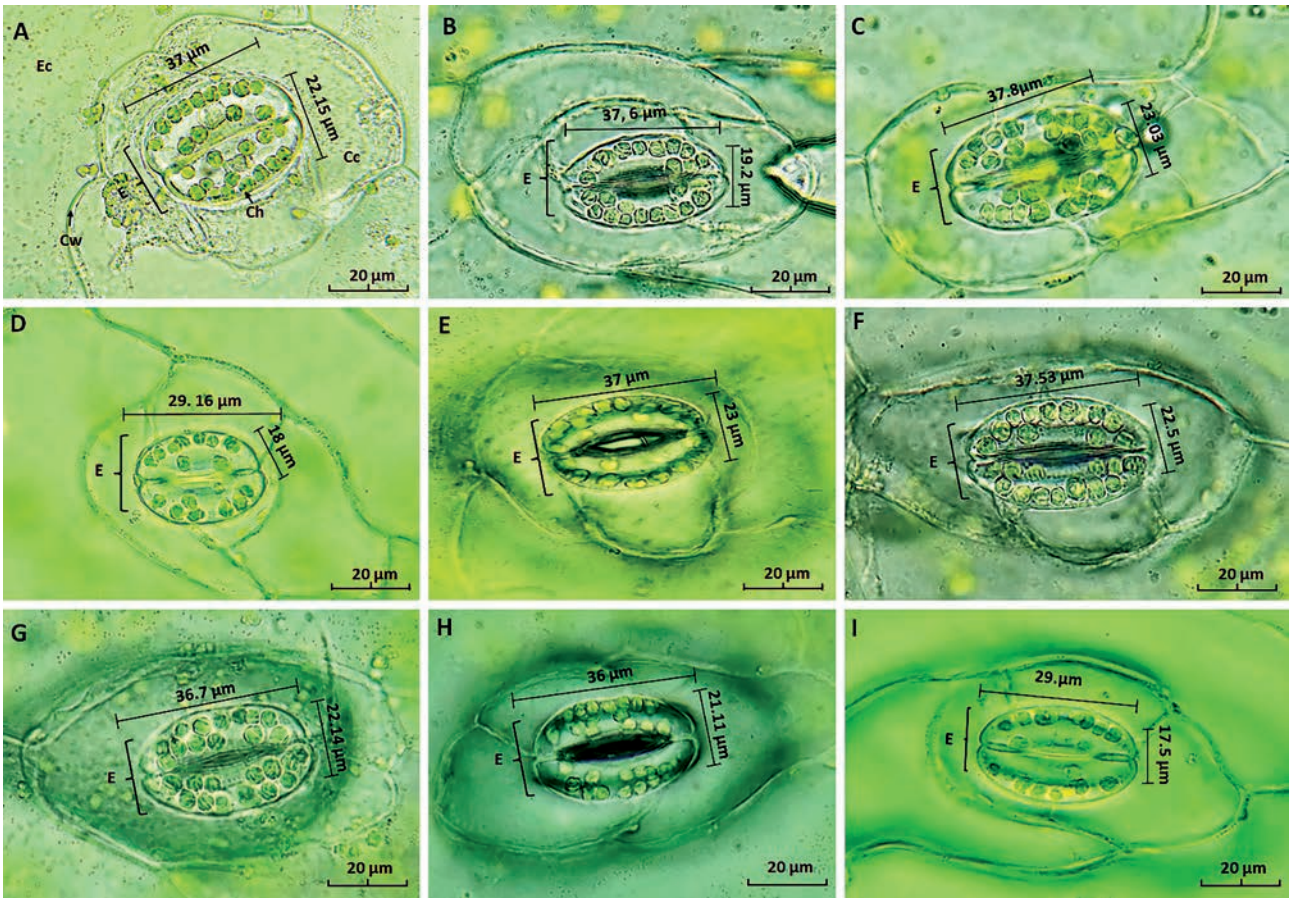

Figure 3 Stomata characteristics of *Kalanchoe tubiflora* (Harv.) Raym.-Hamet leaf blade's abaxial epidermis. (A) T1 stoma: 26 CL. (B) T2 stoma: 25 CL. (C) T3 stoma: 29 CL. (D) T4 stoma: 18 CL. (E) T5 stoma: 25 CL. (F) T6 stoma: 26 CL. (G) and (H): T7 stomata: 26 CL. (I) T8 stoma: 19 CL. Total magnification 1000×. Bar scale = 20 µm. Cc: companion cell; Ec: epidermal cell; Ch: chloroplast; E: stoma; Cw: cell walls

observing the great difference between the T1 and T7 plant height, leaves number, foliar thickness, foliar volume and number of seedlings with the rest of treatments (Fig. 2), the direct effect that colchicine has on the morphology could be confirmed (Salazar et al. 2018); in that way, the use of 0.15 % for 48 h, 0.1 % and 0.15 % for 72 h caused a considerable increase in foliar thickness and 91.7, 78.7 and 100 % (respectively) of tetraploid cells in *Aloe vera* (Molero et al. 2018). Similarly, 3 species of kumquats submitted to colchicine produced thicker, significantly rounder and heavier leaves (Nukaya et al. 2019). Next, when comparing the use of 0.05 % and 0.025 % for 24 h (T2 and T3), likewise, the use of 0.1 % and 0.05 % for 48 h (T5 and T6), it is observed that even doubling the colchicine concentration it does not achieve a significant difference between most of the variables of these treatments, which is similar to Gallone et al. (2014) findings, where treatments with 500 and 1000 µM of colchicine caused an increase in the foliar length and width in *Hebe* Comm ex Juss plants, without significant differences among them.

The increase in the plant organs size due to a polyploid gene expression, is a sample of the evolutionary capacity, due to the plant manages to successfully overcome the different physiological difficulties conferred by the genome multiplication (Grant 1981, Barker 2013, Husband et al. 2013, González et al. 2016). Being able to find several copies of the same gene, becoming paralogical genes (Unver et al. 2017). This phenomenon is considered as an adaptive pro-

Table 3. Stomatic characteristics. Stomatal index, length, width and number of chloroplasts by stomata.

Treatments	Stomatal index	Stomatal length (µm)	Stomata width (µm)	Chloroplasts per stoma
T1	29.3±0.25^{a}	37±0.12^{a}	21.97±0.48^{c}	25.6±1.2^{a}
T2	23.8±1.02^{b,c}	37.16±0.69^{a}	20.12±1.6^{c}	25.4±0.7^{a}
T3	24.2±0.3^{b,c}	37.78±0.17^{a}	23.01±0.3^{b}	28.8±1.3^{b}
T4	10.3±0.9^{d}	28.96±0.27^{b}	18.06±0.08^{a}	18.0±0.9^{c}
T5	22.8±0.35^{c}	37.14±0.3^{a}	22.7±0.44^{c}	25.4±1.1^{a}
T6	24.2±0.1^{b,c}	37.49±0.05^{a}	22.4±0.1^{c}	26.2±1,5^{a}
T7	28.6±0.6^{a,b}	36.94±0.7^{a}	22.05±0.67^{c}	28.6±1.6^{b}
T8	14.6±0.5^{d}	28.66±0.5^{b}	17.5±0.5^{a}	18.4±0.9^{c}

The means ± SD values with different letter of each column show statistically significant differences, according to Tukey´s test ($P \leq 0.05$). SD = standard deviation.

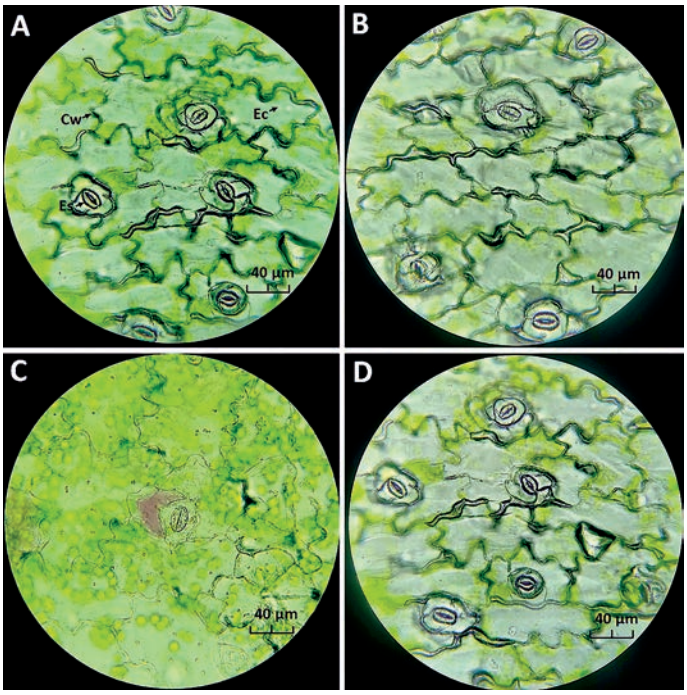

Figure 4 *Kalanchoe tubiflora* (Harv.) Raym.-Hamet leaf blade´s abaxial epidermis (A) T1 Stomatal index. (B) T2 Stomatal index. (C) T4 Stomatal index. (D) T7 Stomatal index. Total magnification 400x. Bar scale = 40 µm. Ec: epidermal cell; Es: Stomata; Cw: cell wall

cess (Quiriban & Cardozo 2017), as happened in *Lippia alba* (Mill.) N.E. Br. ex Britton & P. Wilson polyploid plants which increased up to 11 times the amount of proline in their leaves (Freitas 2018). Which is essential to maintain homeostasis (Ragagnin et al. 2014, Freitas 2018), also to guarantee the protein structures and membranes integrity (Da silva et al. 2013, Taiz et al. 2014, Zandalinas et al. 2017, Freitas 2018), to stabilize DNA as well as to promote growth (Rejeb et al. 2014, Núñez-Vázquez et al. 2018).

Regarding the seedlings production (T1, T6 and T7), there is similarity with Salazar et al. (2018) findings in *Kalanchoe daigremontiana* Raym.-Hamet & H. Perrier plants exposed to 0.025 % for 24 h (7 seedlings), 0.1 % for 48 h (4 seedlings) and 0.025 % for 48 h (19 seedlings). These offspring obtained from the eighth week, inherited the parents morphological and stomatal characteristics. Accordingly, it is known that the *Kalanchoe* genus exhibits very long juvenile phases, such as six years for *Kalanchoe prolifera* Raym.-Hamet (Zeevaart 1985). Currey & Erwin (2011) demonstrated that the culmination of the juvenile phase is one of the important factors that influence the *Kalanchoe beauverdii* Raym.-Hamet, *K. beharensis* Drake, *K. fedtschenkoi* Raym.-Hamet & H. Perrier, *K. longiflora* Schltr., *K. marmorata* Baker, *K. marnieriana* H. Jacobsen, *K. streptantha* Baker, *K. tomentosa* Baker and *K. viguieri* Raym.-Hamet & H. Perrier, reproduction. In turn, it is known that gibberellin acids (GA) can effectively shorten the duration of the juvenile phase in this genus (Sgamma 2017, Chang & Huang 2018), depending then of endogenous signals to change from vegetative development to reproductive development (Noy-Porat et al. 2009, Tan & Swain 2006, Erwin 2006, Nilsson & Weigel 1997, Huang & Chu 2012). In synthesis, it is considered that the appearance of seedlings with the colchicine use is the product of phytoregulators increasing promoted by a possible polyploidy.

It is important to highlingh that the use of 500 mg L^{-1} of colchicine for 36h in *Cucumis sativus* L. explants resulted in 61.54 % of polyploidy, as well as a significant increase in the plant morphology (Ebrahimzadeh et al. 2018). According to Hannweg et al. (2016) the most important advantage of induced polyploidy is that the obtained plants tend to have better morphological characteristics such as height, larger size of leaves, rhizome or root. It also increases the biomass, the photosynthetic capacity, the fruits and seeds size (Urwin 2014). In this study, highly significant differences were registered ($P \leq 0.05$: Tukey HSD) in plants treated with colchicine, which represents a very strong indication of polyploidy (Sadat et al. 2017, Hannweg et al. 2016, Urwin 2014, Salazar et al. 2018).

Stomatic characteristics

It is possible to determine the ploidy level, through the stomata's size and density (Foschi et al. 2013, Beck et al. 2003, Liu et al. 2007, Vandenhout et al. 1995, Gallone et al. 2014). Thus, the stomatal index can be used to distinguish diploid plants from polyploid plants (Khazaei et al. 2010, Zhang et al. 2008, Yang et al. 2006) and tetraploids from hexaploids (Aryavand et al. 2003). In this order of ideas, it can be observed in the stomatal index, that the treatments T1 and T7 maintain the tendency to provoke improvements compared to the rest of the treatments, in addition the remarkable difference of the treatments T2, T3, T5 and T6 is evident compared to treatments T4 and T8 (control), which had the lowest values. This differs from what was observed by Xiang et al. (2019) in tetraploid plants of *Platycodon grandiflorum* A. DC. obtained by exposure to colchicine. These plants showed a lower stomatal index than the control; however, they showed greater stomatal width and length compared to diploid plants. It is similar to what was obtained by Salazar et al. (2018) in *Kalanchoe daigremontiana* plants with a higher stomatal index of 22.5 (0.1 % per 24 h) and a value of 11.78 in the treatment with deionized water for 48 h (control). On the other hand, these results differ from those presented by Silva (2018), where *Citrullus lanatus* (Thunb.) Mansf. plants were exposed to 0.1 % and 0.2 % of colchicine for 24 h and 48 h without significant differences with the control treatments in the mitotic index. Likewise, the stomatal index tetraploid plants *Trachyspermum ammi* induced with colchicine, decreased in contrast to the diploid plants from the control (Sadat et al. 2017).

The use of colchicine significantly increased the stomata length compared to the control treatments (T4 and T8). However, no significant differences were observed between colchicine treatments (T1, T2, T3, T5, T6 and T7), this is similar to the results obtained by Sadat et al. (2017), where, the *Trachyspermum ammi* plants exposure to colchicine caused a significant increase in the stomata length compared to the control plants. In the same way, *Pogostemon cablin* Benth tetraploid plants exhibited larger stomatal size than diploid

plants (Widoretno 2016). Similarly, in a study conducted by Gallone et al. (2014) in "Oratia Beauty" *Hebe* plants with different ploidy level, induced with colchicine (500 and 1000 µM) the stomata length was significantly higher in tetraploids compared to diploids.

The results about the stomata width, indicate that the control treatments (T4 and T8) maintain the lowest values trend, it can also be seen that the exposure to 0.025 % for 24 h caused a greater increase in stomatal width, concordant with the high number of larger stomata, obtained in *Glycyrrhiza glabra* L. seedlings treated with 0.1 % colchicine for 24 h and *Carthamus tinctorius* L. seedlings treated with 0.03, 0.05 and 0.1 % colchicine (Moghbel et al. 2015). On the other hand, these results differ from those presented by Vardar et al. (2017), where *Helianthus annuus* L. outbreaks exposed to colchicine at 0.4 % for 12 h the thorn width decreased a 12.74 % and a 19.69 % in the treatment with 0.6 % for 12 h.

According to Dhooghe et al. (2011) and Xu et al. (2018), the amount of chloroplasts in the guard cells can be used as an indirect way to indicate polyploid plants, in this case T3 present the highest number of chloroplasts per stomata, thus, it maintains the tendency to improve the size of the stoma, it is also important to point out that the T4 and T8 treatments maintain the tendency to show the lowest values of all stomatal characteristics (Table 3), these data agree with those presented by Xu et al. (2018) in *Populus* plants (*in vitro*) treated with 30 mg of colchicine, which chloroplasts average number and foliar morphology significantly increased in the tetraploids versus the diploids. Finally, there are several authors who support the use of colchicine to increase the ploidy of different plant species (Widoretno 2016, Surochita & Debasree 2018), due to the use of this achieves cytogenetic characteristics increasing, which explains, the increase in foliar morphology and chlorophyll content (Allario et al. 2011, Gao et al. 2016). In this study, an increasing in stomatal morphology and characteristics was confirmed, as mentioned by Wang et al. (2019) which stands that the use of colchicine plays an important role in plant breeding because polyploid plants show better characteristics related to growth, yield and quality.

CONCLUSION

The application of colchicine in *Kalanchoe tubiflora* seedlings at 0.025 % for 48 h and 0.1% for 24h significantly increased morphological features such as plant height, length, width, thickness and leaves number, foliar volume and number of seedlings produced. Thus, it is demonstrated that the use of colchicine in the *K. tubiflora* plant favors its biomass and growth increasing in a short time. In the same way, the use of colchicine at 0.025% for 24h significantly increased the stomatal characteristics. Therefore, the importance of comparing morphological and stomatal analysis is observed. In addition, there is a need to carry out additional studies, such as flow cytometry to specifically determine the ploidy level of *K. tubiflora* medicinal plant.

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