https://doi.org/10.48048/tis.2023.6597

Effect of Colchicine on Morphological and Anatomical Traits of *Gymnocalycium mihanovichii* (Frič & Gürke) Britton & Rose

Natthakitti Boonyawiwat[1], Tanapon Siritrakulsak[1], Chadaporn Senakun[2] and Kriangsuk Boontiang[1,*]

[1]Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Maha Sarakham 44150, Thailand
[2]Walai Rukhavej Botanical Research Institute, Mahasarakham University, Maha Sarakham 44150, Thailand

([*]Corresponding author's e-mail: kriangsuk.b@msu.ac.th)

Received: 9 January 2023, Revised: 27 February 2023, Accepted: 24 March 2023, Published: 5 April 2023

Abstract

Cacti are economic ornamental plants whose value varies with market trends. Recently, cacti with new morphologies compared to the original plant have decreased in popularity. Colchicine is a tricyelic alkaloid substance, It is an inhibitor of mitosis thus preventing the formation of microtubuli and the mitotic spindle. This research investigated the effect of colchicine on inducing morphological and anatomical traits in *Gymnocalycium mihanovichii* using a 2-factor factorial completely randomized design (CRD) The first factor was the duration of the dipped stem at 24 and 48 h, while the second factor was the colchicine concentration at 0.25 and 0.5 %. Results indicated that G. *mihanovichii* treated with colchicine at different concentrations and dipping durations showed statistically significant differences in plant height, stem diameter, number of spines and root length. *G. mihanovichii* treated with 0.50 % colchicine for 48 h developed a new morphological trait called spineless cactus. Occurrences of larger sized stomata with lower frequency per unit area were statistically significantly different after treatment with 0.25 % colchicine for 24 h. Maximum length of stomatal guard cells was 50.33 μm.

Keywords: Cactus, Colchicine, Stomata, Spineless, Morphological trait

Introduction

Cacti belong to the family Cactaceae, which comprises 127 genera with 1,896 recorded species having varied morphological characteristics of stem, shape, spines, and flowers with beautiful colors [1,2]. Most cacti are xerophytes and occur naturally in Mexico, the Central Andes of Peru and Bolivia, eastern Brazil, and the Southwestern United States [3]. Cacti are slow-growing plants that thrive in arid areas. They have become popular ornamental plants in the home and workplace, with 130 species imported and exported as ornamentals in 182 countries [4,5]. Some species of cactus are cultivated as food for consumption of fruit and as crops for animal husbandry [6-8].

Gymnocalycium mihanovichii (Frič & Gürke) Britton & Rose is a species of cactus native to North Paraguay. It belongs to the genus *Gymnocalycium* and is also known as "Gymno" or "Chin Cactus", with about 70 species in the subfamily Cactoideae [3]. *G. mihanovichii* has a distinctive variety of morphological characteristics including globose to short cylindrical with the apex depressed, ribs rounded, aeroles rather large, spines variable and the flowers diurnal, subapical, funnel-shaped, and colorful (creamy-white, yellow, pink and red). The fruit is globose to elongated, fleshy, dehiscent laterally and contains many small round seeds [1]. This species is very popular among cactus lovers, comprising over 55 % of purchases compared to other taxa [9]. The Red Hibotan or Hibotar cultivars are varied mutants that lack chlorophyll, showing orange, red or yellow pigmentation. Currently, *G. mihanovichii* is the most valuable cactus species, both in the Thai market and trade worldwide.

Polyploidy is a most important pathway for evolution that can cause reproductive isolation and spontaneous speciation. Polyploidization of plants occurs in nature as a slow and gradual process. Colchicine has been used to induce rapid polyploidization in several plants such as orchids, roses, lilies, *Chrysanthemum*, succulent plants, and cacti [10]. Colchicine blocks the metaphase of the cell cycle, preventing microtubule formation during cell division. This inhibits the separation of chromosomes during

the anaphase stage. Polyploidy plants have more than 2 complete sets of chromosomes. The polyploidy level is mutated by colchicine, and this induces different morphological and physiological traits, stomatal size and stomatal density as an indirect method, and chromosome number as a direct method in breeding programs [10,11]. Polyploid induction in cacti using colchicine can create new traits and add value to the plant. However, consumer trends in the attraction of new plant morphologies or different forms of existing traits vary. This study developed new traits in *G. mihanovichii* using different colchicine concentrations and dipping durations. Mutations of morphological and anatomical traits linked with polyploidization were also investigated.

Materials and methods

Plant material and polyploidy induction

G. *mihanovichii* young plants, form Natta Catus Limited Partnership with diameters 2 - 2.5 cm were used for polyploidy induction following a 2-factor factorial in completely randomized design (CRD) consisting of 5 replicates as the control and 2 different colchicine concentrations of 0.25 and 0.50 % for plants dipping at 24 and 48 h. About 1/8 part of the plant stem were dipped in aqueous colchicine and kept in the dark at room temperature. A stock solution of 1 % (w/v) colchicine was prepared by dissolving 1 g of colchicine (C9754, Sigma-Aldrich) in 100 mL of sterile distilled water. The stock solution was diluted to achieve treatment concentrations at 0.25 and 0.50 %. After colchicine treatments, the plants were thoroughly rinsed 3 times with sterile distilled water and air-dried at room temperature. The plants were then transferred to individual pots (4-inch diameter) containing garden soil, volcanic rock, and coconut flakes (1:1:1) and watered every 5 days. All plants were maintained under shade in the nursery house at the Natta Cactus Limited Partnership, Kantharawichai district, Maha Sarakham Province and the survival rate for each treatment was recorded.

Observation of morphological and anatomical traits

Morphological characteristics of diploid and tetraploid plants were compared for height, stem diameter, number of ribs, number of spines and length of the root at 180 days. A Venier scale with an accuracy of 1 mm was used to measure plant height and stem diameter. Root length was also measured to an accuracy of 1 mm. The numbers of ribs and spines were counted accurately. To observe the anatomical traits, stems of *G. mihanovichii* were cut into small pieces of 0.5×1 cm^2 area and a razor blade was used to scrape off the outer skin tissue. The plant pieces were immersed in 5 % Clorox for 7 to 10 min and then in sterile distilled water for 5 min. Samples were then placed into an object glass and observed under a light microscope (Zeiss, Axio). The stomata guard cells length and stomata density were measured. Twenty stomata guard cell length was measured for each treatment. On each slide, along a diagonal transect of the peel, 4 stomata were measured for guard cell at 400x magnification. The stomata density estimated from the stem surface was measured as the mean number of stomata in 1 mm^2, and stomata in 10 light microscopic were counted for each treatment.

Statistical analysis

SPSS version 29.0 software was used to statistically analyze the data. Data were evaluated by analysis of variance, with significance assessed by Duncan's multiple range test (DMRT). Statistically significant difference was considered at p-value < 0.01, with results displayed in Tables.

Results and discussion

The survival rate of *G. mihanovichii* decreased following colchicine exposure, as shown in **Table 1**. Plants treated at 0.25 % colchicine with dipping duration of 24 and 48 h had survival rates of 80 and 60 %, respectively while plants treated at 0.50 % colchicine with dipping duration of 24 and 48 h had survival rates of 40 %. Results indicated that higher colchicine concentrations reduced the survival rate. A previous report, concerning ornamental plant inductions by applying colchicine, concluded that over the last 15 years, the dipping methods used ranged from 0.015 % for 3 days in Wishbone flower (*Torenia fournieri*) to 1 % for 24 h in Lily (*Lilium*). While, colchicine concentration for seed treatment usually ranges from 0.1 - 0.8 %, high dose cause malformation and reduces the production of tetraploid plants [11]. Therefore, plant survival rates varied depending on plant morphology and physiology. As colchicine is highly toxic to plants, therefore, low doses with prolonged exposure periods are considered reliable to reduce its toxic effect and increase the polyploid production rate [11].

Table 1 Survival rate percentages of *G. mihanovichii* 60 days after treatment with different colchicine concentrations and dipping duration time.

Colchicine concentration (%)/dipping duration time (h)	Survival rate (%)
0.00 (control)	100
0.25 % colchicine for 24 h	80
0.25 % colchicine for 48 h	60
0.50 % colchicine for 24 h	40
0.50 % colchicine for 48 h	40

Growth and morphological characteristics of *G. mihanovichii* after treatment with colchicine at different concentrations and dipping durations were observed, with results shown in **Table 2**. Plants treated with colchicine at 0.00 % (control), 0.25 and 0.50 % showed average increase in plant height and stem diameter from 3.01 cm (control) to 4.12 cm (0.50 % colchicine), and 4.06 cm (control) to 4.55 cm (0.50 % colchicine), respectively. Findings concurred with studies on *Dendranthema indicum* var. *aromaticum* which is tetraploid plants developed larger, thicker leaves, greater flower diameter, more epidermis hairs but shorter plant height than diploid plants [12], and also found in tetraploids of *Platanus acerifolia* which show a more compact growth habit and broader and thicker leaves [13]. These changes resulted from induced polyploidization by colchicine, with enlarged and increased numbers of plant cells due to increased amounts of gene. This increased plant size is called "gigas effect in polyploid" [10].

The number of spines decreased after treatment with colchicine from 5.5 (control) to 1.60 (0.50 % colchicine), while root length decreased from 7.04 cm (control) to 3.79 cm (0.50 % colchicine) with statistically significant difference. The number of ribs in the plant decreased after 0.25 % colchicine treatment, while plants treated at 0.50 % showed increased number of ribs (**Table 2**). Stem diameters of *G. mihanovichii* dipped for 24 and 48 h showed statistically significant decreased values for all parameters, except for the number of ribs (**Table 2**). Therefore, the dipped duration time had no effect on the rib. It may be more time to observe changes in the stem morphology of this plant.

Several previous reports noted greater thickness of leaf and higher number of flowers with increased diameter and weight in polyploidy plants [10,11], while tetraploids of *Platanus acerifolia* showed decreased plant height and number of branches [13]. Similarly, in this study, decreases in plant height and number of spines were recorded in *G. mihanovichii*. This new characteristic would be commercially beneficial and demand high prices in the cactus market because it is rarely found in nature. Root length was also shorter and suitable for planting in a small pot.

Interaction between colchicine concentration and dipping duration on morphological characteristics of *G. mihanovichii* showed that plant height, stem diameter, number of spines and root length all presented statistically significant differences, except for the number of ribs (**Table 2**). Number of spines and root length were significantly reduced on average from 5.40 (control) to 0.80 (0.50 % colchicine for 48 h) and 7.02 cm (control) to 2.12 cm (0.50 % colchicine for 24 h), respectively (**Table 2**). Remarkably, plants treated with 0.50 % colchicine for 48 h developed a new morphological trait called spineless cactus (**Figures 1** and **2**). *G. mihanovichii* can be induced to develop new characteristics using colchicine such as variegated stem color, spineless, cristata and monstrosities. These traits may increase the market value of the cacti both domestically and abroad.

Table 2 Effect of different colchicine concentrations and dipping durations times on the morphological characteristics of *G. mihanovichii.*

Factor	Plant height (cm)	Stem diameter (cm)	No. of ribs	No. of spines	Root length (cm)
Colchicine concentration (%)					
0.00 (control)	3.01^{c}	4.06^{b}	8.50^{b}	5.50^{a}	7.04^{a}
0.25	3.68^{b}	4.55^{a}	6.30^{c}	3.80^{b}	6.66^{b}
0.50	4.12^{a}	4.49^{a}	9.40^{a}	1.60^{c}	3.79^{c}
F-test	**	**	**	**	**

Factor	Plant height (cm)	Stem diameter (cm)	No. of ribs	No. of spines	Root length (cm)
Dipping duration (h)					
24	3.96[a]	5.11[a]	8.13	4.00[a]	5.42[b]
48	3.24[b]	3.62[b]	8.00	3.26[b]	6.24[a]
F-test	**	**	ns	**	**
Colchicine conc. and dipping duration					
0.00, 24 h	3.18[d]	4.06[c]	8.60	5.40[a]	7.02[a]
0.00, 48 h	2.84[e]	4.06[c]	8.40	5.60[a]	7.06[a]
0.25, 24 h	4.12[b]	5.56[b]	6.40	4.20[b]	7.12[a]
0.25, 48 h	3.24[d]	3.54[d]	6.20	3.40[c]	6.20[b]
0.50, 24 h	4.60[a]	5.72[a]	9.40	2.40[d]	2.12[d]
0.50, 48 h	3.64[c]	3.26[e]	9.40	0.80[e]	5.46[c]
F-test	**	**	ns	**	**
C.V. (%)	1.90	2.23	6.60	14.21	1.44

Means within treatment combinations with different superscripts are significantly different by DMRT.
ns = non-significant; ** significant difference ($p \leq 0.01$)

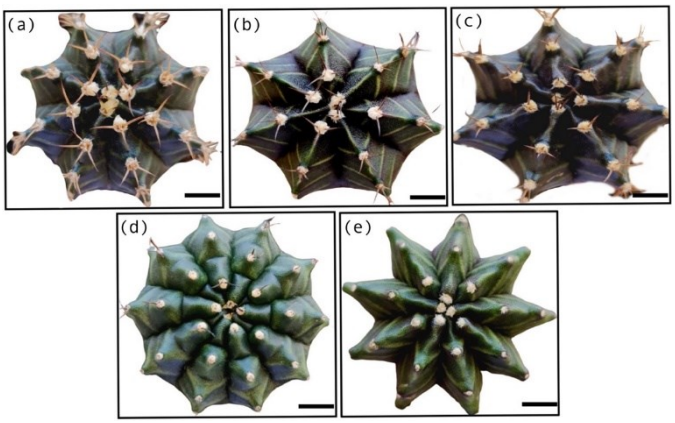

Figure 1 Effect of colchicine concentrations and dipping durations on morphological characteristics of *G. mihanovichii*. (a) Control, (b) Plants treated with 0.25 % colchicine for 24 h, (c) Plants treated with 0.25 % colchicine for 48 h, (d) Plants treated with 0.50 % colchicine for 24 h, and (e) Plants treated with 0.50 % colchicine for 48 h. Scale bar = 1 cm.

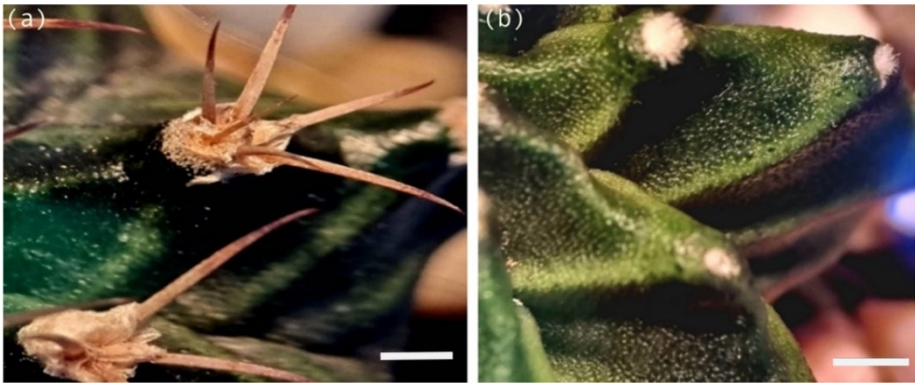

Figure 2 Effect of different colchicine concentrations and dipping durations on morphological characteristics of *G. mihanovichii*. (a) Control 5 - 6 spines, (b) Spineless in colchicine concentration 0.50 % for 48 h. Scale bar = 1 cm.

The epidermis of the stem of *G. mihanovichii* was observed. Both types of stomata were found distributed in the tissue. In anisocytic stomata (**Figure 4(a)**), one of the subsidiary cells surrounding the stomata is smaller than the other epidermal cells, while in paracytic stomata (**Figure 4(b)**), the 2 subsidiary cells are parallel to the long axis of the guard cells. The control plant showed distinctly sunken stomata or hidden stomata. A sunken stoma is a small pit embedded into the leaf or stem layers of cacti rather than on the epidermis surface. This helps to protect against escaping water vapor from air currents, thereby decreasing water loss from the leaf and, hence decreasing transpiration. Sunken stomata are one of the adaptations of a plant to preserve water and are generally found in xerophytes that have adapted to desert and arid environments. In this study, plants treated with higher concentrations of colchicine and longer dipping duration had a thinner epidermis, while the stomatal level was shallower than the control. Therefore, plants treated with colchicine may be suitable for the development of ornamental plants that can be grown in houses. The thin outer tissue and shallower position of the stomata allow the plant to adapt and release water normally [14].

The stomatal characteristics of *G. mihanovichii*, including length of the guard cells and density of stomata, were measured for each treatment. Results for different colchicine concentrations and dipping durations were statistically significantly different, as shown in **Table 3**. Mean length of stomatal guard cells ranged from 36.28 to 50.33 µm, with density of stomata ranging from 13.70 to 23.25 mm^{-2}.

Stomatal guard cell length and stomatal density have been observed at the polyploidy level as an indirect method in several plants [10,11]. Length of stomatal guard cells significantly increased from the control. Plants treated with 0.25 % colchicine for 24 h had maximum length of stomatal guard cells at 50.33 µm, while the control had minimum length of 36.28 µm. Stomatal guard cell density at 0.25 % colchicine for 48 h was significantly different from the control and other treatments.

G. mihanovichii treated with colchicine at different concentrations and dipping durations showed significantly different larger sized stomata at lower frequency per unit area. All plants treated with different colchicine concentrations had significantly different stomatal guard cell densities from the control (21.30 mm^{-2}), *in* particular plants treated with colchicine at 0.25 % for 24 h (13.85 mm^{-2}), 0.50 % for 24 h (13.70 mm^{-2}) and 48 h (14.75 mm^{-2}). Stomatal guard cell density can be a promising target for improving leaf photosynthesis in plants (**Figure 3**). Photosynthesis fitness can be estimated from variance in the stomatal density of leaves in diverse agricultural environments such as drought, salinity stress heat stress and precipitation change. Findings were consistent with previous reports in tetraploid plants such as Orchid Vanda Hybrid [15], *Catasetum pileatum* [16], other ornamentals [11], and horticultural plants [10]. Morphological and anatomical characteristics can be analyzed as good indicators to confirm the conversion of polyploidization [17].

Table 3 Effect of different colchicine concentrations and dipping durations on stomatal characteristics of *G. mihanovichii*.

Colchicine concentration (%)	Dipping duration (h)	Stomatal guard cell length (µm)	Density of stomatal guard cells (mm^{-2})
0 (control)	24	36.28^{d} (± 2.56)	21.30^{b} (± 2.66)
	48	37.08^{d} (± 2.76)	21.70^{b} (± 2.13)
0.25	24	50.33^{a} (± 2.11)	13.85^{c} (± 3.25)
	48	36.72^{d} (± 3.42)	23.25^{a} (± 1.85)
0.50	24	48.06^{b} (± 2.15)	13.70^{c} (± 2.96)
	48	41.19^{c} (± 3.88)	14.75^{c} (± 3.19)
F-test		**	**
C.V. (%)		14.99	27.22

Means within treatment combinations with different superscripts are significantly different by DMRT.
** significant difference ($p \leq 0.01$)

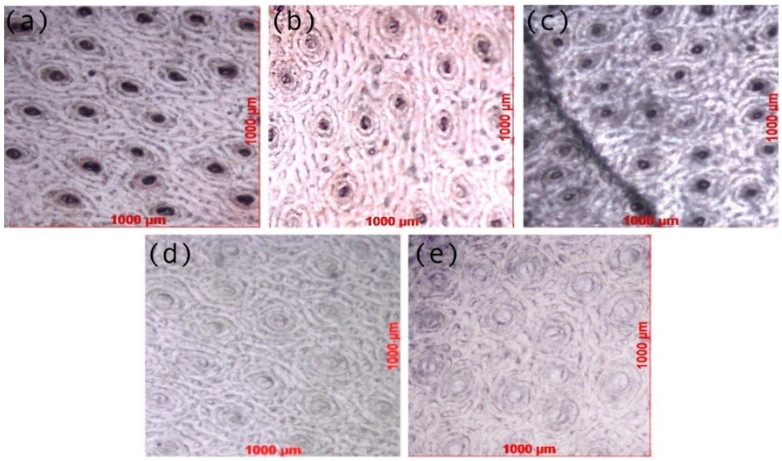

Figure 3 Effect of different colchicine concentrations and dipping durations on stomatal characteristics of *G. mihanovichii.* (a) Control, (b) Plants treated with 0.25 % colchicine for 24 h, (c) Plants treated with 0.25 % colchicine for 48 h, (d) Plants treated with 0.50 % colchicine for 24 h, and (e) Plants treated with 0.50 % colchicine for 48 h.

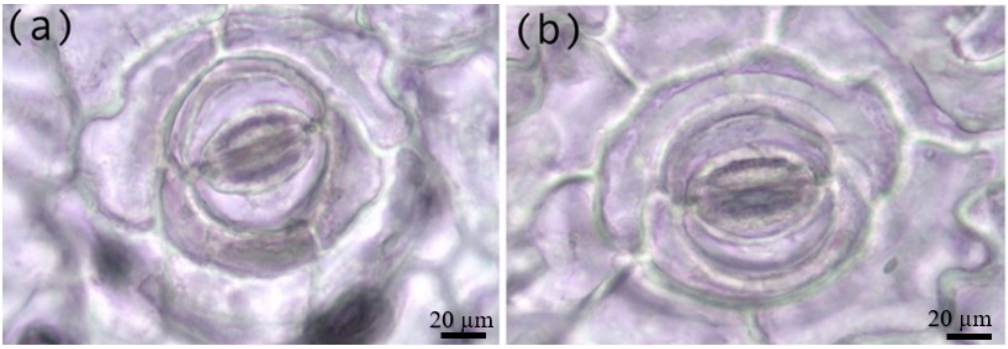

Figure 4 Stomatal types of *G. mihanovichii* in colchicine concentration 0.50 % for 48 h. (a) Anisocytic stomata, (b) Paracytic stomata. Scale bar = 20 µm.

Conclusions

Morphological and anatomical characteristics of polyploidy in *G. mihanovichii* were distinct from the diploid level after colchicine treatment at different concentrations and dipping durations. Differences included increased plant height and stem diameter, with decreased number of spines and root length. A new trait called spineless after treatment with 0.50 % colchicine for 48 h can add commercial value to the cactus. Higher colchicine concentrations impacted stomatal size and density, with larger sized stomata at lower frequency per unit area. Knowledge gained from this study will assist in the breeding of ornamental plants and increase current understanding of levels and patterns of plant breeding.

Acknowledgement

This research project was financially supported by Mahasarakham University, Thailand.

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