

In vitro polyploidization and greenhouse development of polyploids of ornamental Mandevillas

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Abstract

The induction of autopolyploid plants is a valuable strategy for genetic improvement and the development of new cultivars of flowers and ornamental plants. Efficient protocols for obtaining autopolyploids in the genus *Mandevilla* represent useful tools for enhancing traits of horticultural interest for the floriculture market. This study aimed to develop an *in vitro* methodology for polyploidy induction using colchicine applied to three *Mandevilla* genotypes *in vitro*. The highest polyploidization frequency was observed in genotype MD1287 (57.1%), followed by MD1480 (50.0%) and MD1296 (20.0%). Fisher's exact test revealed a highly significant effect of colchicine dose on polyploidization frequency ($p < 0.001$), with no polyploid plants obtained in the untreated control (0.00%) compared to a 44.1% polyploidization frequency at the 0.05% dose. No significant differences in polyploidization frequency were found among genotypes ($p = 0.252$) or between exposure times, 24 h versus 48 h ($p = 1.000$). Colchicine treatment induced significant developmental effects, evident from the *in vitro* culture stage and persisting after acclimatization *ex vitro*. These findings underscore the potential of polyploidization as a strategy to enhance the ornamental value of *Mandevilla* and provide insights for future applications in breeding programs for this genus.

Introduction

Floriculture is among the fastest-growing industries within the horticulture sector, a trend associated with improvements in living standards (Manikas et al., 2019). In this context, the use of new groups of species with ornamental potential involves the establishment of germplasm banks, domestication, and genetic breeding programs through hybridization to commercial cultivation standards (Botelho et al., 2015; Li et al., 2022).

The genus *Mandevilla* comprises species distributed throughout the American continent, with a climbing or semi-climbing habit as its main characteristic for cultivation. These plants prefer well-lit locations, and well-drained soil rich in organic matter and clay, and are primarily marketed as potted plant (Royal Horticultural Society, 2024; Flora e Funga do Brasil, 2024) due to its tubular flowers, predominantly colored white, yellow, orange, pink, and red,. In the international market, *Mandevilla* has remained a stable product in terms of sales volume, with the average price ranging from 4.85 to 5.86 euros between 2017 and 2020 (Ibraflor, 2023). At a single flower center in Netherlands —Plantion—revenue generated from *Mandevilla* sales increased by 50% between 2017 and 2020, reaching €900,000 in 2020 (AIPH, 2022).

Despite its significant economic importance, only a few breeding programs worldwide are responsible for the genetic base of *Mandevilla* cultivars released in local and international markets. Similar to other groups of ornamental species, conventional breeding prevails through the use of controlled crosses between plants with superior traits or specific characteristics of interest. The resulting hybrids are cultivated and selected for their superior and innovative characteristics from F1 populations (Costa, 2018). Key traits of interest includes high-yielding inflorescences with larger flowers, more compact plants suitable to cultivation 12 and 15 cm pots, as well as greater tolerance to climatic conditions and resistance to diseases (Sattler et al., 2016; Mori et al., 2016; Castillo et al., 2020; Gao et al., 2021; Yan et al., 2022; Xiao et al., 2025).

However, the use of biotechnological tools may represent an alternative for accelerating breeding programs, obtaining genetic material distinct from those derived via conventional methods, or even increasing genetic variability within germplasm banks (Thriveni et al., 2024). Polyploidization combined with *in vitro* environmental control and micropropagation techniques represents a valuable tool to increasing the efficiency of obtaining solid autopolyploid plants (Iiyama et al., 2024; Asrandina et al., 2025; Silveira et al., 2015). Autopolyploids are important for restoring fertility and can increase compatibility among genotypes previously incompatible for hybridization (Rieseberg and Willis, 2007; Han et al., 2025).

Colchicine, used in the polyploidization process, is an alkaloid extracted from the plant *Colchicum autumnale*. Its action inhibits the polymerization of tubulin—a structural protein involved in the formation of the mitotic spindle during cell division—thereby arresting metaphase and preventing the division of both the cell and the duplicated genetic content (Esmaeili et al., 2020). However, even after colchicine treatment, not all treated tissues become polyploidized. Thus, it is necessary to select the polyploidized tissues and discard those that remained non-polyploidized, in order to determine the polyploidization efficiency of a protocol (Silva et al., 2022; Rezende et al., 2020). One of the most used methods to establish ploidy and DNA content in plants is flow cytometry, due mainly to the rapid results and to non-destructive sampling (Sliwinska et al., 2022).

Multiple factors are associated with polyploidization efficiency, the main ones being: species, genotype, and ploidy of the original tissues; the type of treated tissue; the concentration and exposure time to colchicine; pre-culture and *in vitro* proliferation time, among others (Bharati & Severova, 2025; Trojak-Goluch et al., 2021; Karami et al., 2019; Wu et al., 2022).

In the case of the genus *Mandevilla*, few studies have focused on evaluating the potential use of autopolyploidization in promising genetic material for flower cultivation. Costa (2018), in a study on the induction of autotetraploids in *Mandevilla bahiensis* under *in vitro* culture, adopted a factorial experimental design with three colchicine concentrations—0.0% (control), 0.025%, and 0.05% w/v—and three exposure times of nodal segments to the antimitotic agent in liquid culture medium (8, 16, and 24 hours). The results showed that the highest percentage of induced tetraploid plants was obtained with the 0.05% colchicine dose combined with 24-hours of exposure. Furthermore, the plants exhibited flowers with larger and thicker petals, wider and thicker leaves (the "Gigas" effect), and higher chlorophyll A and B indices, and increased stomatal size.

Given the need to expand *in vitro* polyploidization protocols, the objective of the present study was to establish an efficient methodology for *in vitro* cultivation, involving polyploidy induction using colchicine in three *Mandevilla* genotypes with ornamental potential (1480, 1296, and 1287). Additionally, the effects of colchicine on the vegetative and reproductive development of *Mandevilla*, both *in vitro* and *ex vitro* conditions were also evaluated, providing a comprehensive comparison between autopolyploidized and control plants.

Materials and Methods

Plant materials, cultivation conditions and colchicine treatments

Three hybrid genotypes of *Mandevilla* were used, whose parental crosses were kept confidential by the company that supplied the genetic material. The codes used to identify the genotypes were MD 1480 (medium growth habit, yellow flowers turning to salmon), MD 1296 (compact growth habit, orange flowers), and MD 1287 (compact growth habit, yellow flowers).

Young shoots from adult plants of these genotypes were collected and packaged in plastic bags containing moistened cotton for transport. For *in vitro* establishment, the explants underwent surface cleaning with hydrophilic cotton and an antiseptic solution (Riohex® 2% chlorhexidine digluconate), followed by rinsing under running tap water for approximately five seconds. Leaves and stems were then sectioned to reduce explant size. Inside the laminar flow hood, explants were immersed in 70% ethanol for thirty seconds, followed by immersion in a solution prepared with 75% commercial bleach (2.0–2.5% active chlorine) under agitation for twenty minutes. After three consecutive rinses in autoclaved deionized water, the explant ends damaged by contact with the disinfection solution were trimmed, and explants were introduced into modified MS medium (Murashige & Skoog, 1962) supplemented with 1.0 mg L⁻¹ 6-benzylaminopurine (BAP). The inoculated segments measured 1.0 cm in length. Every thirty days, shoots were individualized and subcultured in the same medium until 50 plants were obtained for each treatment.

Nodal segments, each containing one lateral bud, were separated from these plants and placed into 250-mL Erlenmeyer flasks containing 125 mL of free-sucrose liquid MS medium. Colchicine solution was added to three of the six flasks containing liquid medium, establishing the treatment with 0.05% of the antimitotic agent.

Colchicine was added via cold sterilization, using a sterilization filter (EMD Millipore Millex™). All Erlenmeyer flasks, with and without colchicine, were kept in the dark at 25 ± 2°C under constant agitation at 100 rpm using an orbital shaker. The nodal segments remained in the liquid media for two immersion periods: 24 and 48 hours.

After the exposure time of each treatment, the nodal segments were removed from the liquid media (with and without colchicine) and rinsed three times consecutively in sterile deionized water. The segments were then subcultured on solid MS medium supplemented with 1.0 mg L⁻¹ BAP for 30 days under a photosynthetic photon flux density (PPFD) of 112.6 µmol m⁻² s⁻¹, a 16-h photoperiod, and an average temperature of 25 ± 2°C. Controls (without colchicine) were maintained under identical conditions.

After sixty days of *in vitro* culture, the plants were removed from the agar, transplanted into paper pots containing substrate, and kept in a mini tunnel with weekly irrigation. This stage was carried out at the cultivar-supplying company, Terra Viva®. After six weeks of cultivation in the mini tunnel, the plants were transplanted into 12 cm pots (12-cm top diameter, 1-L substrate capacity) containing pine bark substrate (pH 5.5). They were maintained in a Poly Shade greenhouse (8 m × 40 m) equipped with 40% Aluminet® shading and a 150-micron light-diffusing plastic cover, fertilized with NPK 12-8-14, and irrigated twice a week for two months. After two months of cultivation in size-12 pots (when plants reached approximately 20 cm in height), evaluations of the material were initiated. The stages of *in vitro* establishment, treatment, acclimatization, and evaluation are briefly illustrated in Fig. 1 below.

In vitro Evaluations

Explant survival and rate of new shoots per nodal segment were evaluated during *in vitro* culture, in order to monitor the *in vitro* development of the materials. Explant survival was determined by direct counting of the number of living explants relative to the total number of inoculated explants at thirty and sixty days after inoculation. Explants that maintained green color, turgor, and no visible signs of oxidation or contamination were considered surviving explants. The rate of new shoots formation was evaluated by the average number of shoots produced per nodal segment on the same evaluation dates (30 and 60 days). Counts were performed individually for each explant, and data were recorded and used to calculate treatment means.

Evaluations in Potted Plants

Morphophysiological evaluations were performed on potted plants after complete *ex vitro* establishment. The parameters evaluated were related to leaf physiology, leaf morphology, floral morphology, floral colorimetry, photosynthesis, and root morphology, as described below. At this stage, samples were also collected for plant ploidy analysis.

Plant Ploidy

To determine plant ploidy, 10 samples were collected from each treatment, along with 10 control samples from each cultivar. Each sample consisted of a shoot with approximately three pairs of fully developed leaves. Samples were sent twice for ploidy confirmation. Ploidy level was confirmed by flow cytometry, following a protocol based on the OTTO lysis buffers (Otto, 1992). Leaf fragments of approximately 2 cm² were chopped in OTTO-I buffer (50 µg mL⁻¹ RNase; 2 mM dithiothreitol), filtered through a 30 µm nylon mesh, and centrifuged at 100 × g for 5 min. The pellet was resuspended in OTTO-I buffer and incubated for 10 min. Nuclear staining was performed using OTTO-II buffer containing propidium iodide (75 µg mL⁻¹ PI; 50 µg mL⁻¹ RNase; 2 mM dithiothreitol; 400 mM Na₂HPO₄·H₂O), followed by filtration through a 20 µm nylon mesh and incubation for 30 min in the dark. Samples were analyzed on a BD Accuri C6 flow cytometer (488-nm laser; emission FL2: 615–670 nm and FL3: >670 nm). Plants displaying a DNA content equivalent to twice that of the diploid control were classified as tetraploids, while those displaying two simultaneous peaks were classified as mixoploids. Only histograms with a coefficient of variation (CV) < 5% were included in the analysis.

Leaf Pigments

Leaf chlorophyll content was indirectly estimated via chlorophyll A index, chlorophyll B index, and the chlorophyll A/B ratio, using a CFL1030 Falker portable chlorophyll meter. Readings were taken on the adaxial leaf surface, avoiding veins, under stable lighting conditions. For each plant, three readings per leaf were taken at a standardized position, and the average value was used for statistical analysis.

Stomatal Morphology and Stomatal Density

Stomatal characteristics were evaluated on the abaxial leaf surface using optical microscopy at 400× magnification. To obtain epidermal impressions, clear nail polish was applied to the abaxial leaf surface, allowed to dry, and removed using transparent adhesive tape (cellophane tape), which was mounted on a glass slide for observation. Images were captured using a microscope and analyzed using ImageJ® software. The polar and equatorial diameters of both the stomata and ostiole, as well as stomatal density (expressed as the number of stomata per square millimeter; stomata mm⁻²), were measured. For each plant, 10 random microscopic fields per slide were analyzed. For stomatal characterization, 20 measurements were collected for each parameter.

Leaf Morphology

Leaf dimensions were measured on the 3rd and 4th leaf pairs from the shoot apex, using fully expanded leaves. Leaf length, width, and thickness were evaluated using an MTX® digital caliper. Leaf area was determined using a LI-3100C leaf area meter (LI-COR®), which performs real-time optical scanning to measure area in cm².

Floral Morphology

Floral evaluations were conducted on fully open flowers and floral buds. Using an MTX digital caliper, the length, width, and height of the corolla were measured in open flowers, as well as the length and width of floral buds. Measurements were collected from an average of 5 flowers per treatment (and from polyploids plants after identification) due to the low flowering frequency observed in the plants.

Floral Colorimetry

Petal coloration was quantified using the CIELAB colorimetric system with a CM-26d spectrophotometer (Konica Minolta®). The parameters L* (lightness), a* (green-to-red chromaticity axis), and b* (blue-to-yellow chromaticity axis) were recorded. Readings were taken directly on the petal surface, in triplicate per petal, standardizing the reading area to minimize intra-petal variation. Readings were performed on 5 replicates per treatment, as well as on identified polyploids.

Photosynthetic Parameters

Photosynthetic efficiency was evaluated through three parameters: the potential photochemical efficiency of photosystem II (Fv/Fm), electron transport rate (ETR), and stomatal conductance (gs). Measurements were taken using a LI-600 portable fluorometer (LI-COR®) between 11:00 a.m. and 12:30 p.m. All readings were performed on the leaf lamina (avoiding veins and margins) on the 3rd or 4th leaf pair from the shoot apex.

Root Morphology

Root morphology was evaluated on soil-free roots. Roots were photographed with a digital camera using a standardized scale. The resulting images were analyzed using ImageJ® software with the SmartRoot® plugin, which enables root structure segmentation and quantification. Average root diameter and average

root length were determined. Furthermore, a qualitative analysis was conducted to better understand the changes in root architecture. Only roots for identified polyploids and control plants were evaluated.

Statistical Analysis

All evaluated parameters (except ploidy) were subjected to analysis of variance (ANOVA), and means were compared by the Scott-Knott test at significance levels of $p < 0.001$, $p < 0.01$, and $p < 0.05$, using R software (R Core Team, 2024). Ploidy frequency and efficiency of polyploidization were statistically evaluated using Fisher's exact test, performed with jamovi software (The jamovi Project, 2026).

Results

Successful In Vitro Establishment and Proliferation of *Mandevilla*

Nodal segments enabled the successful *in vitro* establishment of *Mandevilla*, resulting in an efficiency of 95%, 87%, and 92% of explants free of visible contamination in the culture medium for cultivars MD1287, 1296, and 1480, respectively. In the contaminated explants, a high persistence and frequency of bacterial contamination was observed, characterized by a cream-yellowish coloration, especially around the explants, which was common across all three genotypes evaluated. Due to its persistence in these and other inoculated *Mandevilla* genotypes, samples were sent for identification of the observed bacterium.

Bacterial identification was performed using DNA extraction followed by PCR amplification of the 16S rRNA marker; the resulting fragment was subjected to next-generation DNA sequencing (NGS) and compared against a reference database containing over 15,000 bacterial strains. The analysis revealed 96.74% similarity with the genus *Kosakonia*, and subsequent BLAST analysis showed 99.93% sequence identity with *Kosakonia radicincitans*. This species is recognized as an endophytic nitrogen-fixing bacterium that exerts positive effects when inoculated into crop plants, such as tomato (*Solanum lycopersicum*) (Berger et al., 2017; Matared et al., 2026).

Explants free of contamination that initiated shoots from adventitious buds were transferred to fresh culture media for multiplication. Results demonstrated that MS medium supplemented with 1.0 mg L⁻¹ BAP successfully promoted the proliferation of *Mandevilla* explants, producing multiple shoots with proliferation rates of 3:1, 2:1, and 3:1 every 30 days of culture for genotypes MD1287, 1296, and 1480, respectively. No morphological alterations or anomalies were observed in the explants during the culture period. The explants used for this experiment were derived from the third subculture.

In vitro Development Following Colchicine Treatment

Explants treated with colchicine for 24 and 48 h were compared to non-treated control explants in terms of nodal segment survival and the number of new shoots per treated explant. No significant differences were observed among genotypes; therefore, the variables were pooled and analyzed as representative of the genus *Mandevilla* (Table 1). Evaluations were performed 60 days after culture initiation following colchicine treatment.

Table 1
Survival rate of *in vitro* nodal segments following colchicine treatment for 24 and 48 hours

Colchicine		Nodal Segments (explants)	
Concentration (%)	Exposition Time (h)	Survival (%)	Number of New Shoots
0.0	24	100 Aa	2.5 Aa
	48	73.3 Ab	1.9 Ab
0.05	24	77.3 Ba	1 Ba
	48	56 Bb	0.7 Bb
F(Dose)		17.36***	57.10***
F(Time)		24.49***	5.06*
F(Interaction)		1.62	0.21
Means followed by the same letter in the column do not differ significantly from each other by the Scott-Knott test; uppercase letters refer to the dose factor, while lowercase letters indicate the time factor; *, **, *** significant at the 5%, 1%, and 0.1% probability levels, respectively;			

Colchicine concentration and exposure duration significantly affected post-treatment survival and explant development. Control treatments (without colchicine) submerged in liquid medium for 24 h resulted in 100% nodal segment survival, with the number of new shoots ranging from 1.9 to 2.5 shoots per explant. Survival decreased to 77.3% and 56.0% when explants were exposed to 0.05% colchicine for 24 and 48 h, respectively. Treatment without colchicine for 48 h also reduced explant survival to 73.3%, suggesting that prolonged immersion in liquid medium negatively impacts explant viability.

Considering each exposure time, both in the presence and absence of colchicine, at 24 h there was a 23% reduction in survival due to the presence of colchicine, whereas at 48 h a 24% reduction in survival was observed with the use of this alkaloid.

Efficiency of Colchicine in Chromosome Doubling of *Mandevilla*

Fisher's exact test, applied due to the categorical nature of the data (polyploid vs. non-polyploid), revealed a significant effect only regarding whether nodal segments of *Mandevilla* were treated with colchicine or left untreated ($p < 0.01$) (Table 2). A comparison between the control group (0.00% colchicine) and the treated group (0.05% colchicine) demonstrated a significant effect of colchicine on polyploidy induction, yielding a 44.1% frequency of polyploidized plants.

Table 2

Frequency of polyploidization among cultivars and exposure times to 0.05% colchicine in *in vitro* nodal segments of *Mandevilla*, according to Fisher's exact test.

Factor	Category	Diploid (n)	Polyploid (n)	Total (n)	Frequency (%)	Efficiency (%)	p-value (Fisher)
Dose	0.00%	34	0	34	0.0	0.0	< 0.001
	0.05%	19	15	34	44.1	8.2	
Cultivar	MD 1287	6	8	14	57.1	11.46	0.252
	MD 1296	8	2	10	20.0	2.17	
	MD 1480	5	5	10	50.0	10.44	
Exposition time (h)	24 hours	8	8	16	50.0	7.01	0.730
	48 hours	11	7	18	38.9	9.04	
Fisher's exact test was applied due to the limited sample size in some categories. <i>p-values</i> > 0.05 indicate the absence of a statistically significant difference between the compared groups (<i>α</i> = 0.05).							

No significant differences in polyploidization frequency were observed among the evaluated cultivars ($p = 0.252$) or between the tested exposure times ($p = 0.730$).

Ploidy and Flow Citometry

For the cultivar MD 1296, the flow cytometry histograms indicated that the G0/G1 ratio of two plantlets exposed to 0.05% colchicine for 24 h approached nearly 2x the 2C DNA content; however, these specific samples displayed two simultaneous G1 peaks (corresponding to 2C and 4C DNA levels) and were thus classified as mixoploids. A similar response was observed for one plantlet of MD 1480 exposed to 0.05% colchicine for 24 h.

For MD 1287 the flow cytometry histograms evidenced that the G0/G1 peak channel of treated-nine (five derived from 24 hours exposure to colchicine and four derived from 48 hours exposure to colchicine) plantlets corresponded to approximately double in relation to control plantlets. Therefore, the polyploidization procedure was effective, as nine samples exhibited double the nuclear 2C value compared to the control (untreated) plantlets. The control (untreated) and non-polyploidized samples showed a mean nuclear 2C value of 1.26 pg (1C = 0.63 pg; 1C = 614.35 Mbp). The polyploid plantlets, in turn, have a mean nuclear 2C value of 2.69 pg, equivalent to 1C = 1.34 pg and 1C = 1314.68 Mbp.

In the remaining treated samples of MD 1480, four polyploid plantlets were successfully identified based on the doubling of their G0/G1 peak channel relative to the diploid control, confirming effective chromosome duplication for this genotype. As ploidy was inferred from nuclear DNA content without chromosome counting, the plants are referred to as DNA-polyploids (Sliwinska et al., 2022).

Leaf Chlorophyll Content and Photosynthetic Efficiency

Regarding chlorophyll content, significant differences were observed only in chlorophyll 'a' content for a single cultivar (MD1480). Polyploids obtained from treatments with 0.05% colchicine for 24 and 48 h exhibited lower leaf chlorophyll contents compared to non-polyploidized plants. In the other cultivars (MD1287 and MD1296), these differences between polyploids and non-polyploids were not detected. Chlorophyll b contents and the chlorophyll a/b ratio also showed no significant differences when comparing polyploidized plants with non-polyploid counterparts. Photosynthetic efficiency was evaluated by comparing polyploidized and non-polyploid plants using chlorophyll fluorescence and stomatal conductance (gsw). The quantum efficiency of photosystem II (Φ PSII) in MD1287 polyploids was significantly lower (Φ PSII = 0.30–0.31) than in non-polyploid MD1287 controls (Φ PSII = 0.65–0.75). For cultivar MD1296, these differences were substantially milder; however, a reduction in the electron transport rate (ETR) of polyploids was observed in this cultivar compared to non-polyploids (Table 3). For cultivar MD1480, no differences were observed in Φ PSII, but the electron transport rate was also reduced in polyploidized genotypes compared to non-polyploid controls (Table 3). Regarding the gsw, differences were observed only in cultivar MD1287, which exhibited a drastic reduction of gsw in polyploidized individuals ($0.01 \mu\text{mol m}^{-2}\text{s}^{-1}$) compared to non-polyploids ($0.09\text{--}0.15 \mu\text{mol m}^{-2}\text{s}^{-1}$) (Table 3).

Table 3

Chlorophyll a and b content, a/b ratio, and photosynthetic efficiency of polyploids identified in cultivars MD 1480, MD 1296, and MD 1287, resulting from *in vitro* colchicine treatment for 24 and 48 hours

Cultivar	Dose (%)	Exposition Time (h)	Ploidy	Chlorophyll Content			Photosynthetic Efficiency		
				A	B	A/B	gs (mol m ⁻² s ⁻¹)	ΦPSII	ETR (μmol e ⁻ m ⁻² s ⁻¹)
MD 1480	0	24	Control	31.62 a	9.08	3.50	0.04	0.75	42.586 b
		48	Control	32.74 a	9.00	3.69	0.08	0.72	45.352 a
	0.05	24	Polyploid	28.82 b	7.33	4.11	0.03	0.75	38.287 b
		48	Polyploid	29.44 b	10.32	3.77	0.09	0.74	37.965 b
F				7.01***	0.38	2.24	0.78	2.29	6.02**
MD 1296	0	24	Control	39.55	14.62	2.72	0.08	0.77 a	45.350 a
		48	Control	38.06	14.45	2.70	0.09	0.74 b	44.536 a
	0.05	24	Polyploid	38.95	15.28	2.62	0.08	0.75 b	37.967 b
F				1.37	0.49	0.28	0.22	10.91*	11,70**
MD 1287	0	24	Control	36.98	14.99	3.48	0.15 a	0.75 a	47.494
		48	Control	39.32	13.21	2.98	0.09 b	0.65 a	43.819
	0.05	24	Polyploid	38.02	15.53	2.63	0.01 c	0.30 b	49.879
		48	Polyploid	39.06	16.43	2.55	0.01 c	0.31 b	51.521
F				0.59	0.88	2.22	17.81***	29.36***	0.20

Mixoploids and tetraploids were grouped as "polyploid". Means followed by the same letter in the column, within each data block, do not differ significantly from each other by the Scott-Knott test (5% level of significance); *, **, *** significant at the 5%, 1%, and 0.1% probability levels, respectively;

Morphology and Density of Stomata in Leaves

Regarding stomatal morphology, an evaluation of the treated groups in MD1480 revealed that explants exposed to colchicine for both 24 and 48 h exhibited an increase in polar and equatorial stomatal diameters compared to non-treated control groups (Table 4). This pattern is attributable to the higher frequency of polyploidized plants in colchicine-treated groups, given that polyploid plants produced stomata with larger diameters than non-polyploidized ones (Table 5, Fig. 2). Similarly, in MD1287, polyploid plants were found to possess stomata with larger diameters than their non-polyploid counterparts (Table 5).

Table 4

Polar and equatorial diameter of stomata under *ex vitro* conditions, resulting from plant treatment with colchicine for 24 and 48 hours *in vitro*

Stomata Ø (µm)							
Dose (%)	Exposition Time (h)	MD 1480		MD 1296		MD 1287	
		Polar	Equatorial	Polar	Equatorial	Polar	Equatorial
000	24	24.84 Bc	16.34 Bc	30.22 Aa	21.15 Aa	29.90 Ab	21.03 B
	48	25.37 Bc	15.97 Bc	28.92 Aa	20.55 Aa	31.21 Ab	21.17 B
0.05	24	30.28 Ab	18.78 Ab	25.75 Ab	18.48 Bb	33.08 Aa	22.01 A
	48	37.23 Aa	24.78 Aa	30.24 Aa	19.95 Bb	29.97 Ab	21.63 B
F(Dose)		164.74***	127.89***	3.47	32.28***	1.28	4.40*
F (Time)		57.55***	66.91***	3.56	2.29	1.12	0.28
F (Interaction)		19.23***	33.01***	11.69**	12.81***	6.70*	0.72
Means followed by the same letter in the column do not differ significantly from each other by the Scott-Knott test; uppercase letters refer to the dose factor, while lowercase letters indicate the time factor; *, **, *** significant at the 5%, 1%, and 0.1% probability levels, respectively.							

Table 5
Stomatal density and polar and equatorial diameters of stomata in polyploids and diploids

Stomatal parameters						
Cultivar	Dose (%)	Exposition Time (h)	Ploidy	Stomatal density	Ø Polar	Ø Equatorial
MD 1480	0	24	Control	120.71 a	24.84 c	16.34 c
		48	Control	120.71 a	25.37 c	15.97 c
	0.05	24	Polyploid	66.52 b	30.28 b	18.78 b
		48	Polyploid	47.73 c	37.23 a	24.78 a
F				165.79***	80.51***	75.93***
MD 1296	0	24	Control	222.88 a	30.22	21.15 a
		48	Control	210.06 a	28.92	20.55 a
	0.05	24	Polyploid	176.03 b	27.99	19.21 b
F				17.24***	1.99	14.89***
MD 1287	0	24	Control	106.72 a	30.36 c	21.03 b
		48	Control	109.65 a	30.35 c	21.17 b
	0.05	24	Polyploid	73.40 c	39.31 a	24.36 a
		48	Polyploid	89.13 b	35.93 b	24.53 a
F				7.73***	24.70***	12.01***
Means followed by the same letter in the column do not differ significantly from each other by the Scott-Knott test; uppercase letters refer to the dose factor, while lowercase letters indicate the time factor; *, **, *** significant at the 5%, 1%, and 0.1% probability levels, respectively.						

However, interesting, the opposite trend was observed in cultivar MD1296, with polyploidized individuals exhibiting smaller stomata compared with non-polyploids (Table 5). This same stomatal pattern was observed in colchicine-treated groups compared to untreated controls (Table 4). These findings demonstrate that the stomatal response in *Mandevilla* appears to be genotype-dependent, indicating that polyploidization can exert distinct effects across different cultivars.

Leaf and Root Morphology

No statistically significant differences in leaf area were observed between colchicine-treated and non-treated plants for any of the evaluated *Mandevilla* cultivars. However, in two cultivars (MD1480 and MD1287), an increase in leaf thickness was reported in plants treated with 0.05% colchicine, regardless of the exposure duration (Table S5). Furthermore, polyploids exhibited predominantly coriaceous leaves (Fig. 3).

The same pattern was observed when comparing polyploidized plants with non-polyploids, with polyploidized plants displaying greater leaf thickness (Table 6). Regarding the direct comparison between

non-polyploid plants and colchicine-induced polyploids, significant differences in leaf area were detected across all three cultivars: polyploidization resulted in a larger leaf area in two cultivars (MD1296 and MD1287), whereas in MD1480, polyploid plants exhibited a smaller leaf area compared to control plants. This variation was largely accounted for by an increase in leaf width (Table 6) and a more rounded leaf shape in polyploid plants (Fig. 3 below).

Table 6

Leaf Thickness (LT) and Leaf Area (LA) under *ex vitro* conditions, resulting from plant treatment with colchicine for 24 and 48 hours *in vitro*

Leaf Thickness and Leaf Area							
Dose (%)	Exposition Time (h)	MD 1480		MD 1296		MD 1287	
		LT (cm)	LA (cm ²)	LT (cm)	LA (cm ²)	LT (cm)	LA (cm ²)
0.0	24	0.21 B	16.30	0.19	10.14	0.21 B	12.92
	48	0.22 B	16.14	0.23	10.45	0.20 B	12.56
0.05	24	0.29 A	18.33	0.21	10.21	0.26 A	12.03
	48	0.27 A	16.54	0.22	10.03	0.29 A	10.50
F (dose)		19.28***	1.42	0.56	0.91	25.56***	2.02
F (Time)		0.03	0.92	2.12	0.14	0.62	0.83
F (Interaction)		0.07	0.64	0.56	1.76	1.46	0.32
Means followed by the same letter in the column do not differ significantly from each other by the Scott-Knott test; uppercase letters refer to the dose factor, while lowercase letters indicate the time factor; *, **, *** significant at the 5%, 1%, and 0.1% probability levels, respectively;							

There are no significative differences in root length and diameter between polyploidized and non-polyploid plants of *Mandevilla*. The root system showed heterogeneous patterns among the cultivars. In MD 1480, polyploid plants exhibited a visibly more branched root system with a higher abundance of fine lateral roots compared to the diploid control. In MD 1296, polyploid plants lacked a tuberous root, comparing with the diploid control, which displayed a well-defined tuberous root. In MD 1287, no clear visual differences in root morphology were observed between polyploid and diploid plants (Fig. 4 below), with the characteristic tuberous root maintained in both groups.

Flower Size and Color

Variation in floral dimensions in polyploids were subtle, with minimal visual impact, and were restricted to specific polyploids without exhibiting substantial size increases in response to polyploidization (Table 7). The petals of certain tetraploid plants displayed alterations in shape, adopting a circular outline rather than the typical half-moon profile (Figs. 5 and 6 next).

Table 7

Flower length (L), width (W), and height (H), as well as bud length (L) and width (W) under *ex vitro* conditions, of polyploids identified in the MD 1287 cultivar groups treated with *in vitro* colchicine for 24 and 48 hours

Floral parameters							
Dose (%)	Exposition Time (h)	Ploidy	Flower			Bud	
			L (mm)	W (mm)	H (mm)	L (mm)	W (mm)
0.0	24	Control	38.96 b	40.09 b	26.52	36.65 a	5.80 b
	48	Control	40.16 b	41.78 b	26.69	34.73 a	5.51 b
0.05	24	Polyploid	40.17 b	40.78 b	25.02	28.87 b	6.66 a
	48	Polyploid	49.67 a	50.05 a	26.48	28.86 b	6.49 a
F			7.98***	6.03**	2.20	10.72***	5.51**
Mixoploids and tetraploids were grouped as "polyploid". Means followed by the same letter in the column, within each data block, do not differ significantly from each other by the Scott-Knott test (5% level of significance); *, **, *** significant at the 5%, 1%, and 0.1% probability levels, respectively;							

Polyploid individuals of *Mandevilla* MD1287 exhibited a slight decrease in lightness (L, Table 8). The flowers of this variety are predominantly yellow; however, polyploidization also reduced the intensity of the yellow coloration in the flowers (b, Table 8) using CIELAB model.

Table 8

Colorimetric parameters of MD 1287 polyploids derived from plants subjected to different exposure times to colchicine

Colorimetric parameters of Flowers					
Dose (%)	Exposition Time (h)	Ploidy	Flower		
			L	a	b
0.0	24	Control	80.62 a	12.27 a	66.39 a
	48	Control	80.29 a	12.55 a	66.02 a
0.05	24	Polyploid	77.89 b	9.92 b	60.28 b
F			14.33***	6.04**	4.27*
Tetraploids were grouped as "polyploid". Means followed by the same letter in the column, within each data block, do not differ significantly from each other by the Scott-Knott test (5% level of significance); *, **, *** significant at the 5%, 1%, and 0.1% probability levels, respectively.					

For cultivar MD1480, no changes in lightness (L) were observed, retaining the lighter coloration of the petals; however, plants derived from the colchicine treatment exhibited a reduction in yellow color intensity (b), similar to the response observed in MD1287. A distinct response was observed exclusively in cultivar MD1296, which displayed a slight increase in yellow coloration in plants obtained from the 0.05% colchicine treatment compared to untreated controls.

Discussion

The variation in frequency among the tested treatments and cultivars stems from a multifactorial cause and may be associated with antimitotic doses, exposure time, application method, and the species evaluated. In the study by Costa (2018), where colchicina concentrations of 0.025% and 0.05% were tested for 8, 16, and 24 h to induce polyploidy in *Mandevilla bahiensis*, the highest percentage of tetraploids (17%) was achieved with 0.05% colchicine for 24 h of exposure, followed by 0.025% for 24 h (9%). These findings guided the choice of dosage in the present study, expanding the exposure times to the antimitotic agent. Mahanta et al. (2023) evaluated the *in vitro* polyploidization efficiency of three colchicine concentrations (0.1%, 0.25%, and 0.5% w/v) for 4, 6, 8, and 12 h in gerbera shoot tips, obtaining the highest induction efficiency at 0.1% for 4 h, which reinforces the importance of the dose × time relationship.

Although widely used as a chromosome-doubling agent, colchicine is a highly toxic alkaloid that interferes with the cell cycle, potentially compromising explant viability (Manzoor et al., 2023). In the present study, nodal segment survival decreased with increasing exposure time to the antimitotic agent in cultivars MD 1480 and MD 1296, corroborating the pattern described in the literature. Dhooghe et al. (2011) highlighted that lower doses for shorter periods tend to preserve tissue survival but result in a lower polyploid frequency, whereas higher doses and longer exposure times increase the polyploidization rate at the cost of higher mortality and the formation of undesirable ploidy levels, such as octaploids.

This dose–response relationship was manifested differently among the evaluated genotypes. In MD 1296, the 48-h treatment resulted in only 32% survival and an absence of polyploids, indicating that toxicity surpassed the induction capacity in this cultivar. A similar result was observed by Talei and Fotokian (2020) in lemon balm (*Melissa officinalis*), where explants exposed to higher concentrations for longer periods failed to survive the treatment. Cultivar MD 1287 maintained stable survival even under 48 h of exposure. This response is consistent with a potential genotype-dependent sensitivity to the antimitotic agent—even though, in the present study, Fisher's Exact Test indicated no statistically significant difference among cultivars ($p = 0.252$)—a pattern also observed in *Mandevilla bahiensis* by Costa (2018) and in gerbera by Mahanta et al. (2023). These results indicate that optimizing polyploidization protocols must account not only for colchicine concentration and exposure time, but also for the physiological specificities of each genotype.

The approximately two-fold increase in the G0/G1 peak fluorescence in MD 1287 flow cytometry analysis is consistent with successful genome duplication and the evaluation of morphophysiological parameters in polyploids identified by flow cytometry reveals the possibility of using such traits to screen polyploids. However, this was not consistent in all cases, particularly when analyses were performed across treatments rather than among individual plants, alongside the fact that many of these parameters were reduced rather than increased. Therefore, the evaluation of morphophysiological parameters should be approached with caution when used as a polyploid identification method.

The reductions or stability in chlorophyll contents observed in polyploids in the present study contrast with the pattern most frequently described in the literature, where polyploid plants typically exhibit higher chlorophyll contents compared to their diploid counterparts (Mohammadi, Kaviani, and Sedaghatpour, 2021; Vishnupandi et al., 2023). However, this pattern is not absolute. Polyploidy and genome duplication can lead

to the dysregulation of genes controlling chlorophyll biosynthesis, impacting the plant's capacity to produce this pigment (Mohammadi et al., 2023). Furthermore, reduced efficiency in pigment synthesis may be related to physiological stress caused by colchicine and cellular damage resulting from its mode of action (Gaynor, Lim-Hing, and Mason, 2020). Consequently, chlorophyll quantification can be highly variable and less discriminating when used in isolation as an indirect parameter for polyploid identification.

Meanwhile, the heterogeneity of physiological responses among polyploids of the same genotype is consistent with observations in other species. In *Melissa officinalis*, tetraploids exhibited superior photosynthetic efficiency parameters compared to diploids (Bharati et al., 2023), whereas in *Ranunculus auricomus*, natural polyploids demonstrated a greater capacity for oxidative stress regulation under high-light conditions (Ulum, Hadacek, and Hörandl, 2021). The diversity of photosynthetic profiles obtained here may stem from structural leaf changes, biochemical modifications associated with chromosome duplication, and possibly the hybrid nature of the evaluated cultivars, whose combined genomes may amplify response variability to polyploidy (Pelé, Rousseau-Gueutin, and Chèvre, 2018).

Induced polyploidization demonstrated potential for generating new *Mandevilla* genotypes with distinct ornamental characteristics. Polyploids of MD 1480 and MD 1287 displayed leaves with greater thickness and a leathery, glossy appearance—traits associated with increased robustness and post-harvest durability, which are valued attributes in the potted plant market (Sattler, Carvalho, and Clarindo, 2016). Regarding cultivar MD 1287, the average increase in floral corolla size and the altered petal shape—from a crescent to a circular profile—confer a distinct visual appearance and market-differentiating potential for ornamentals. These morphological modifications are consistent with the *gigas* effect, frequently associated with chromosome duplication (Vilcherrez-Atoche, Iiyama, and Cardoso, 2022), representing phenotypic variability of direct interest for ornamental breeding programs. The floriculture market is largely driven by the continuous supply of new genotypes with novel traits (Ibraflor, 2023). *Mandevilla*, established as a stable product in the potted segment with increasing average prices in the international market (AIPH, 2022), lacks genetic diversification, as its cultivar base remains narrow and reliant mostly on conventional breeding (Costa, 2018). *In vitro* polyploidization emerges as a complementary strategy capable of expanding this genetic base, producing individuals with distinct phenotypes and commercial potential without the reproductive barriers inherent to interspecific hybridization.

Regarding the flower color variations observed between polyploid and diploid plants, our findings corroborate the literature, which frequently reports cultivar-dependent effects. In *Hydrangea macrophylla* 'David Ramsey' (Deans et al., 2021), *Melissa officinalis* (Bharati et al., 2023), and different genotypes of *Portulaca grandiflora* (Aisyah et al., 2024), chromosome duplication did not result in significant alterations in flower color. Additionally, reductions in intensity or fading of original coloration have been reported in *Phalaenopsis* spp. orchids (Samanhudi et al., 2023). These results indicate that induced polyploidy does not always translate into increased color intensity, potentially promoting gene silencing, disruptions in enzymatic regulation within metabolic pathways, or cellular dilution effects (Kawakami et al., 2021; Samanhudi et al., 2023). In this regard, the chromatographic parameter results obtained for cultivar MD 1287 reinforce the complex and genotype-specific nature of polyploidization. A limitation of the present study was the absence of flowering in MD 1296 during the evaluation period, which precluded a comprehensive comparison of floral parameters among all three genotypes. In *Dendrobium wardianum* Warner. (Wang et al., 2023) and *Begonia × benariensis*

(Xie et al., 2024), heterogeneous responses to polyploidization among cultivars of the same genus were also reported, with significant variations in morphological and physiological traits depending on the genotype, which is consistent with the results obtained here.

The absence of a consistent pattern of increase in polyploid root parameters was previously described by Kikuchi et al. (2024), who observed in *Arabidopsis thaliana* autopolyploids that root growth in tetraploids was favored by larger cell volume, whereas hexaploids and octaploids exhibited root growth suppression associated with cell-cycle regulation difficulties. Although the polyploids obtained in the present study are tetraploids, cell-cycle difficulties resulting from chromosome set alterations may contribute to the observed lack of root enhancement. In radish, Kim, Lee, and Chae (2022) also reported reductions in root weight and width in tetraploids compared to diploids, attributing this to the slower growth rate characteristic of polyploid plants—a dynamic similarly observed in the overall development of polyploid plants in the present study.

Conclusions

The immersion protocol using 0.05% colchicine for 24 h in liquid MS medium was the most efficient treatment aiming at polyploids in *Mandevilla*. However, the recovery frequency was genotype-dependent, with a lower frequency in cultivar 1287 (20%) and higher rates in the other two cultivars (50–57.1%). Polyploids of cultivars MD1480 and MD1287 exhibited increased leaf thickness, larger stomata, subtle modifications in petal shape, and a reduction in yellow flower intensity—traits of interest for breeding programs—without substantial alterations in primary ornamental characteristics. Indirect morphophysiological parameters (chlorophyll content, stomatal traits, leaf dimensions) commonly employed for polyploid identification should be not precise for screening in *Mandevilla* and differentiation from non-polyploidized, as responses were also genotype-dependent, requiring flow cytometry for confirmation. The polyploids described here in represent basic genetic material for use as parental lines in ornamental *Mandevilla* breeding, aiming to evaluate the generation of novel hybrids in the breeding of this important ornamental species.

Declarations

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Authors contribution

CRedit Author Statement: M.P. Leite: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. J.C. Cardoso: Conceptualization, Supervision, writing – original draft and review & editing. W. Ferreira: analysis, review & editing. E. G. Costa: Conceptualization, resources, review & editing. Vilcherrez-Atoche, J.A. analysis, writing – original draft

Declaration of competing interests

The authors report there are no competing financial or personal interests to declare that could have appeared to influence the work reported in this paper.

The data that support the findings of this study are available from the corresponding author, J.C. Cardoso, upon reasonable request.

Declaration of generative AI use

During the preparation of this manuscript the author(s) used Gemini / Google in order to revise and correct the English language of the manuscript and to provide a schematic figure for demonstration of the methodology used in each step of polyploidization, called graphical abstract (Figure 1). After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article

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Figures

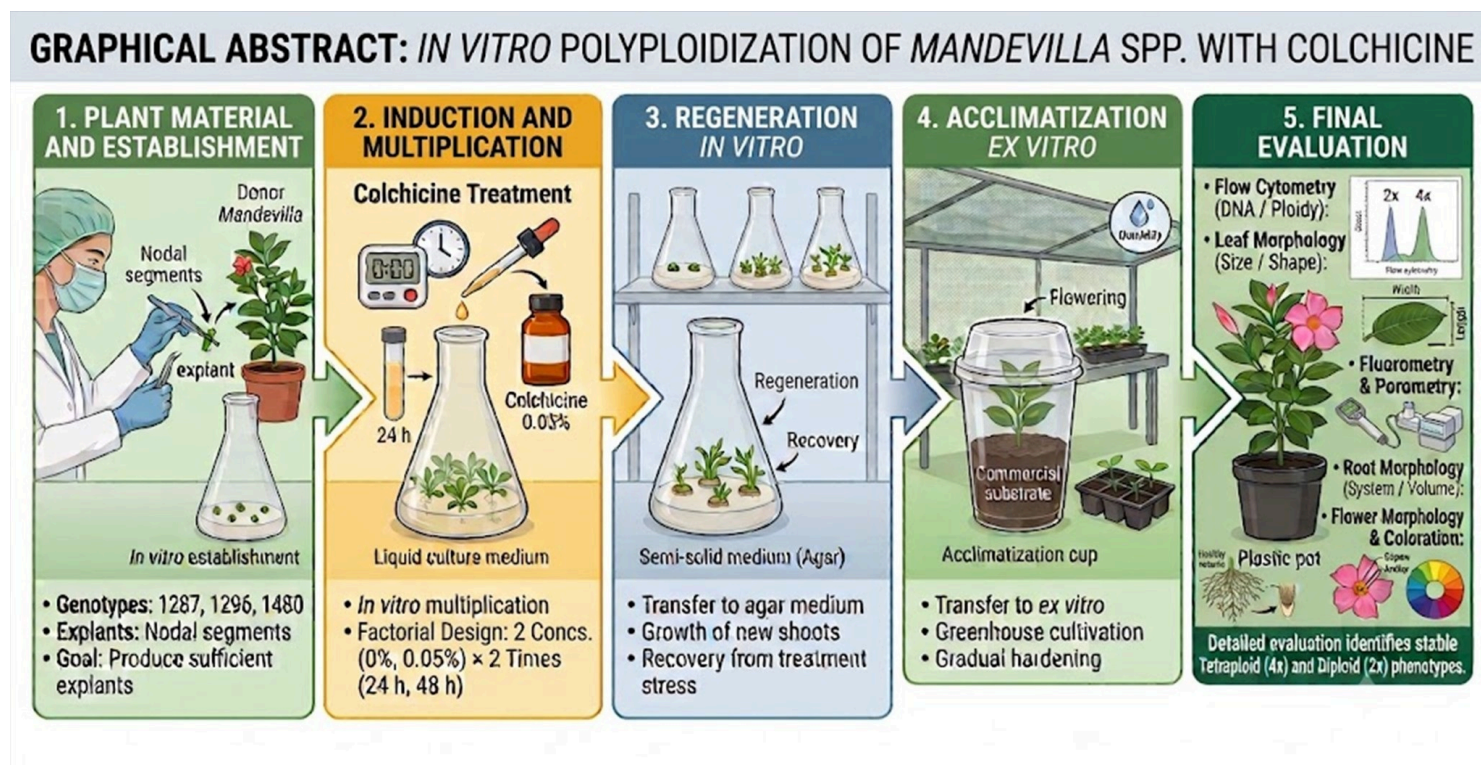


Figure 1

Graphical summary of the treatment and analysis stages.

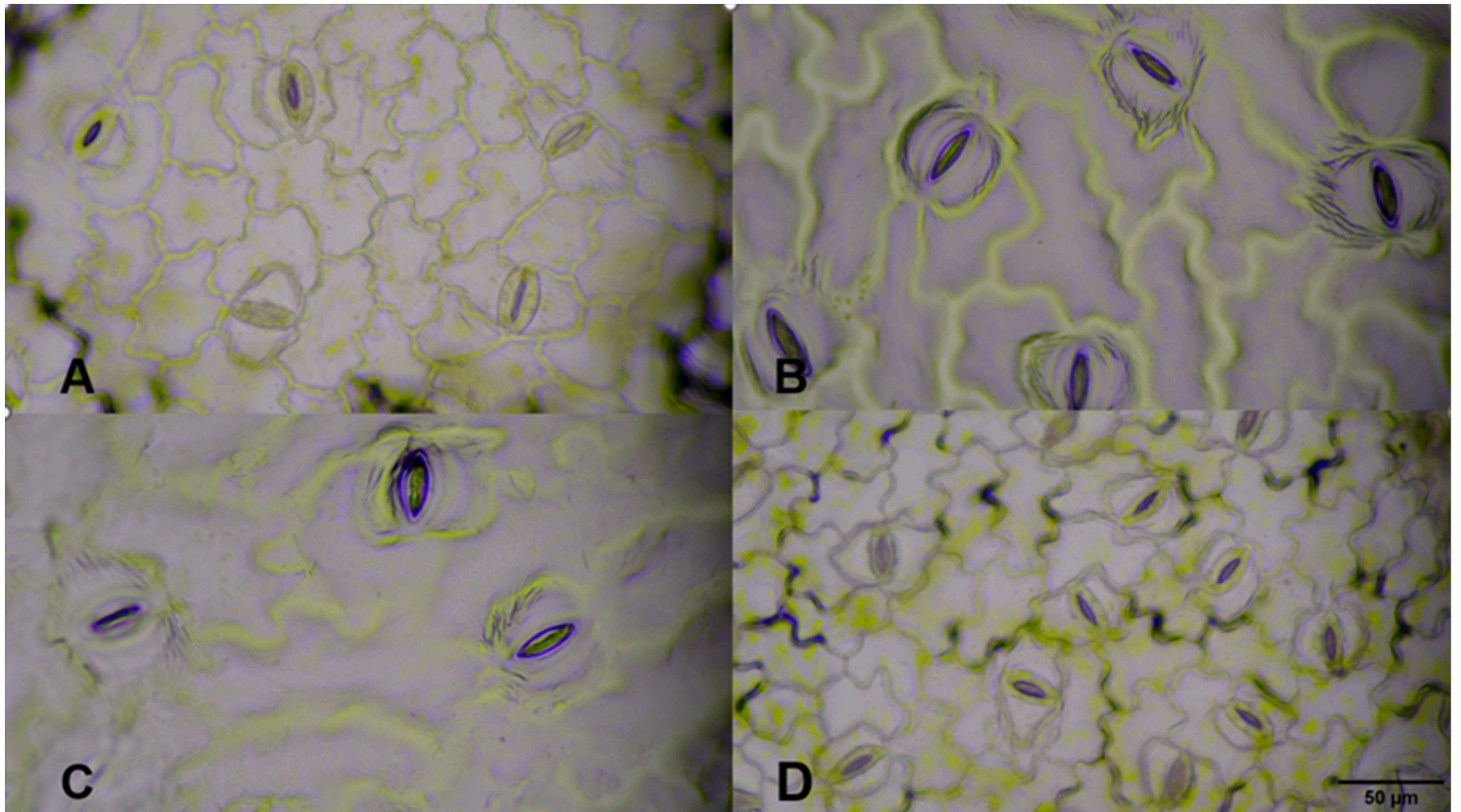


Figure 2

Stomata of cultivar MD 1480 exposed and unexposed to colchicine Stomata of: (A) MD 1480 mixoploid derived from the 0.05% treatment for 24 h; (B) MD 1480 tetraploid derived from the 0.05% treatment for 24 h; (C) MD 1480 tetraploid derived from the 0.05% treatment for 48 h; (D) MD 1480 diploid control.

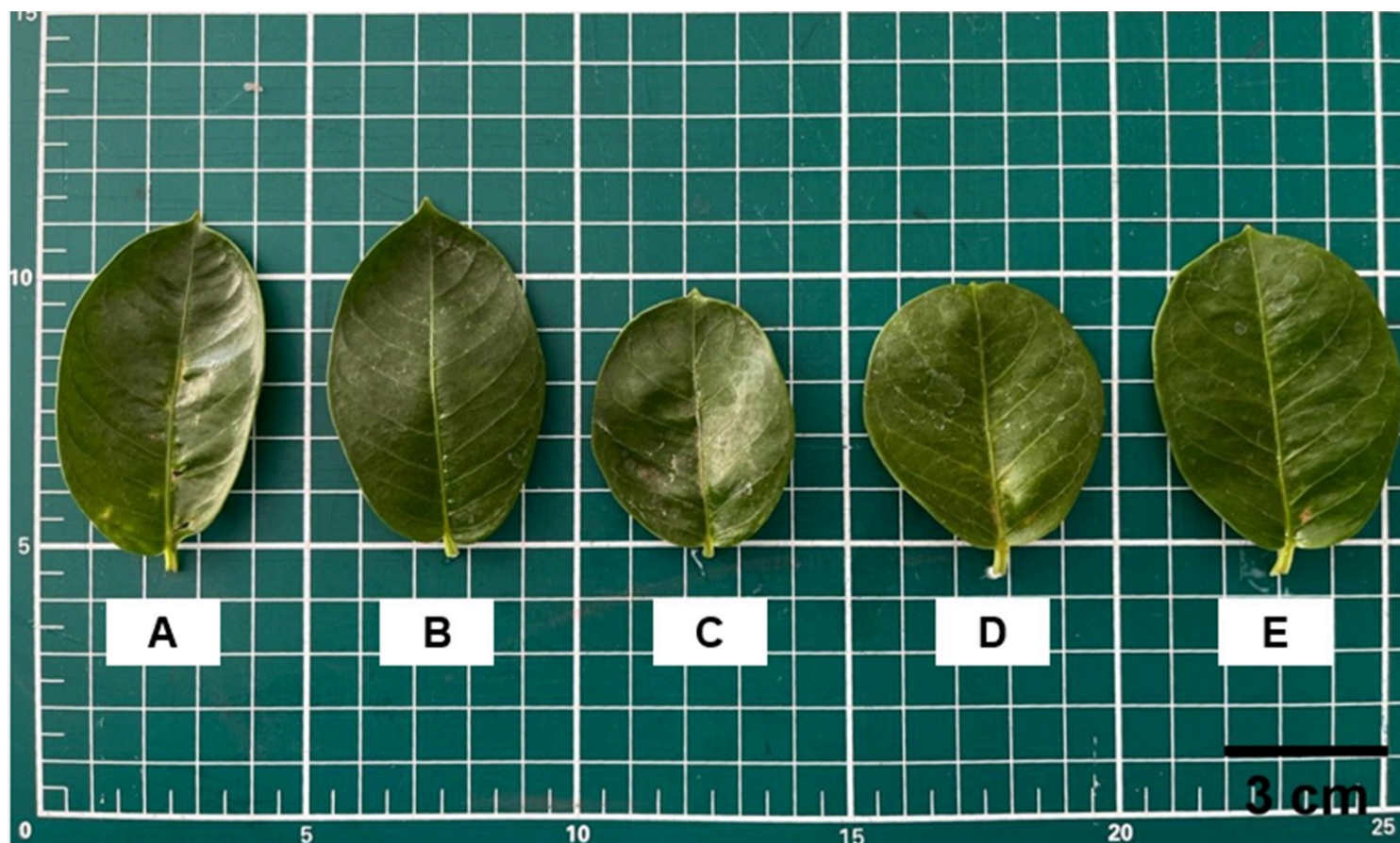


Figure 3

Morphological characteristics of MD 1480 leaves treated or not with colchicine for 48 hours Plant leaves of the MD 1480 cultivar: (A and B) control treatment; (C) Tetraploid 2, derived from the 48-hour colchicine treatment; (D) Tetraploid derived from the 48-hour colchicine treatment; (E) Tetraploid 4, derived from the 48-hour colchicine treatment.

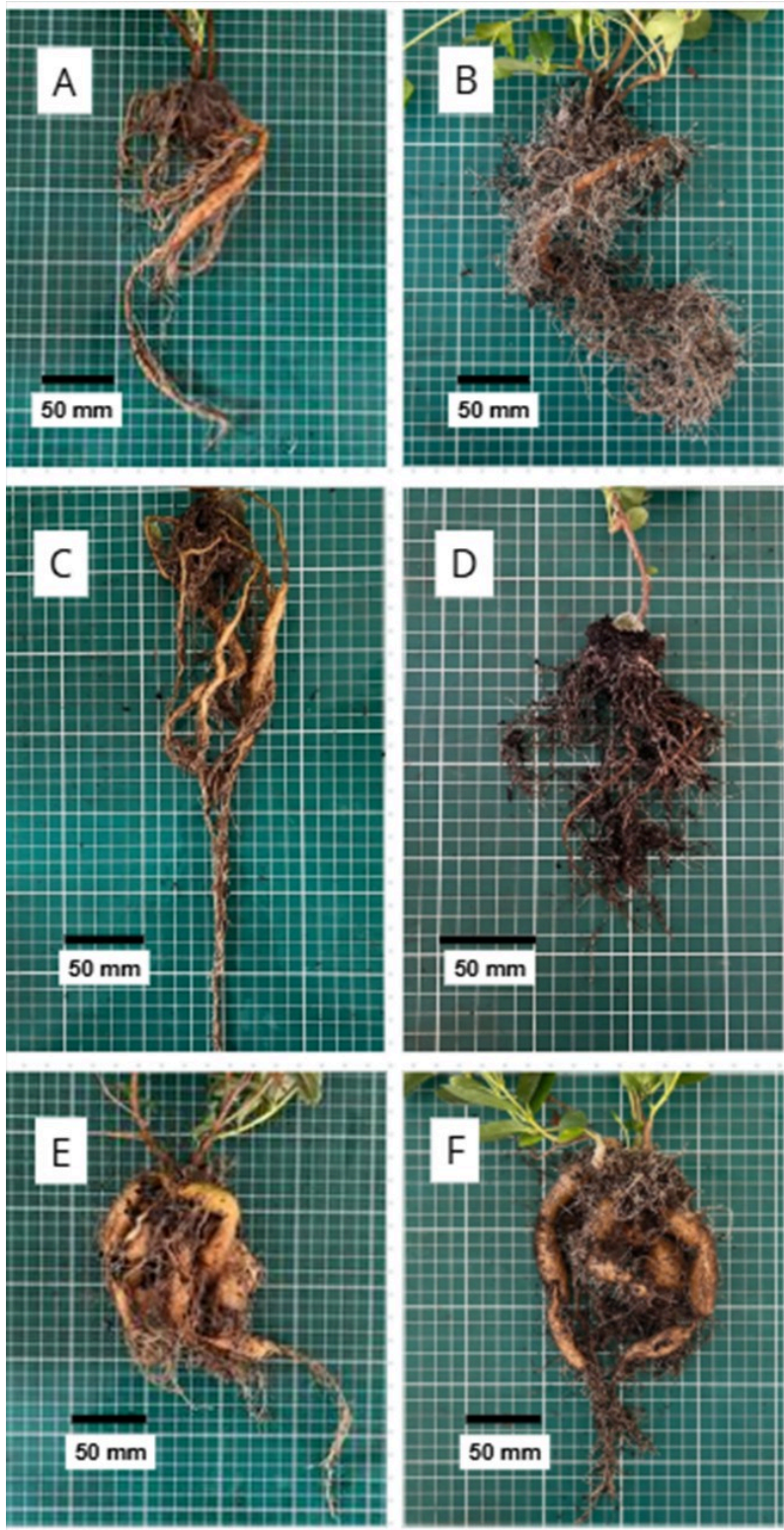


Figure 4

Comparison of roots between diploid and polyploid *Mandevilla* plants (A) Diploid *Mandevilla* 1480, (B) Polyploid *Mandevilla* 1480, (C) Diploid *Mandevilla* 1296, (D) Polyploid *Mandevilla* 1296, (E) Diploid *Mandevilla* 1287, (F) Polyploid *Mandevilla* 1287

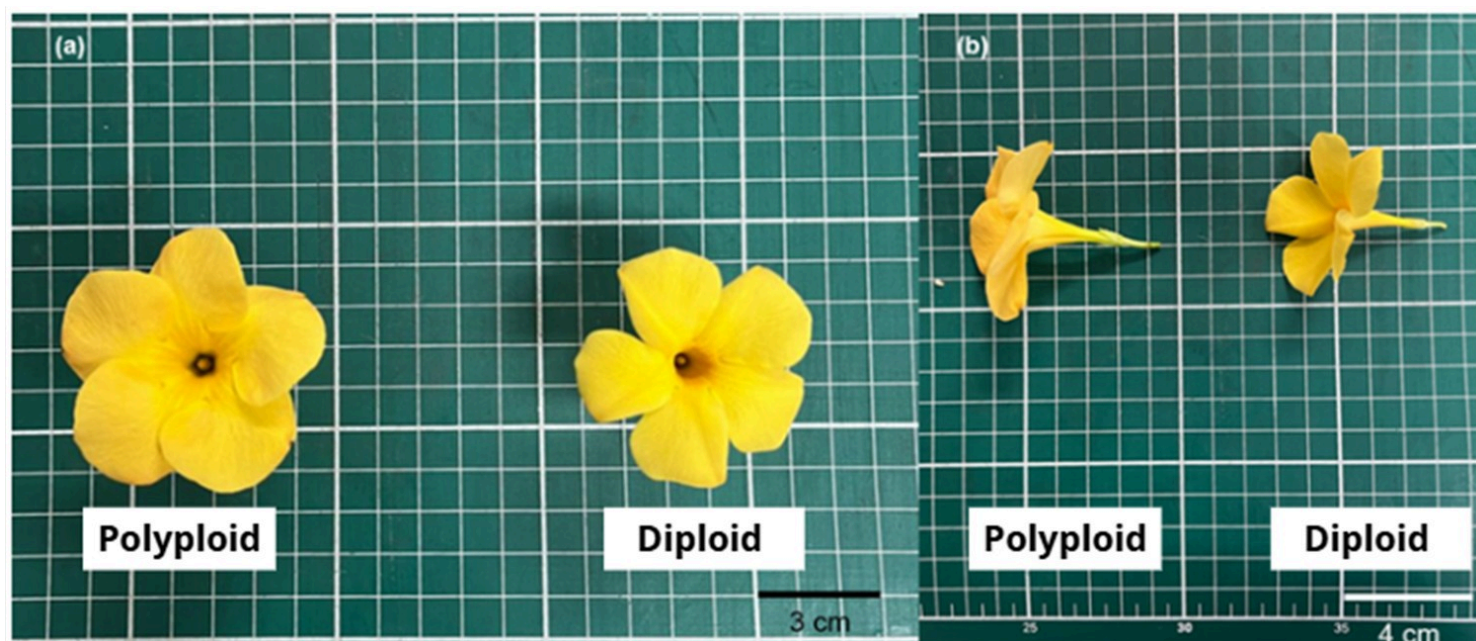


Figure 5

Morphological characteristics of flowers of MD 1287 treated and untreated with colchicine (a) Front view of flowers of the MD 1287 cultivar; (b) Lateral view of flowers of the MD 1287 cultivar – on the left in both images is a tetraploid (tetraploid 3), and on the right is a diploid

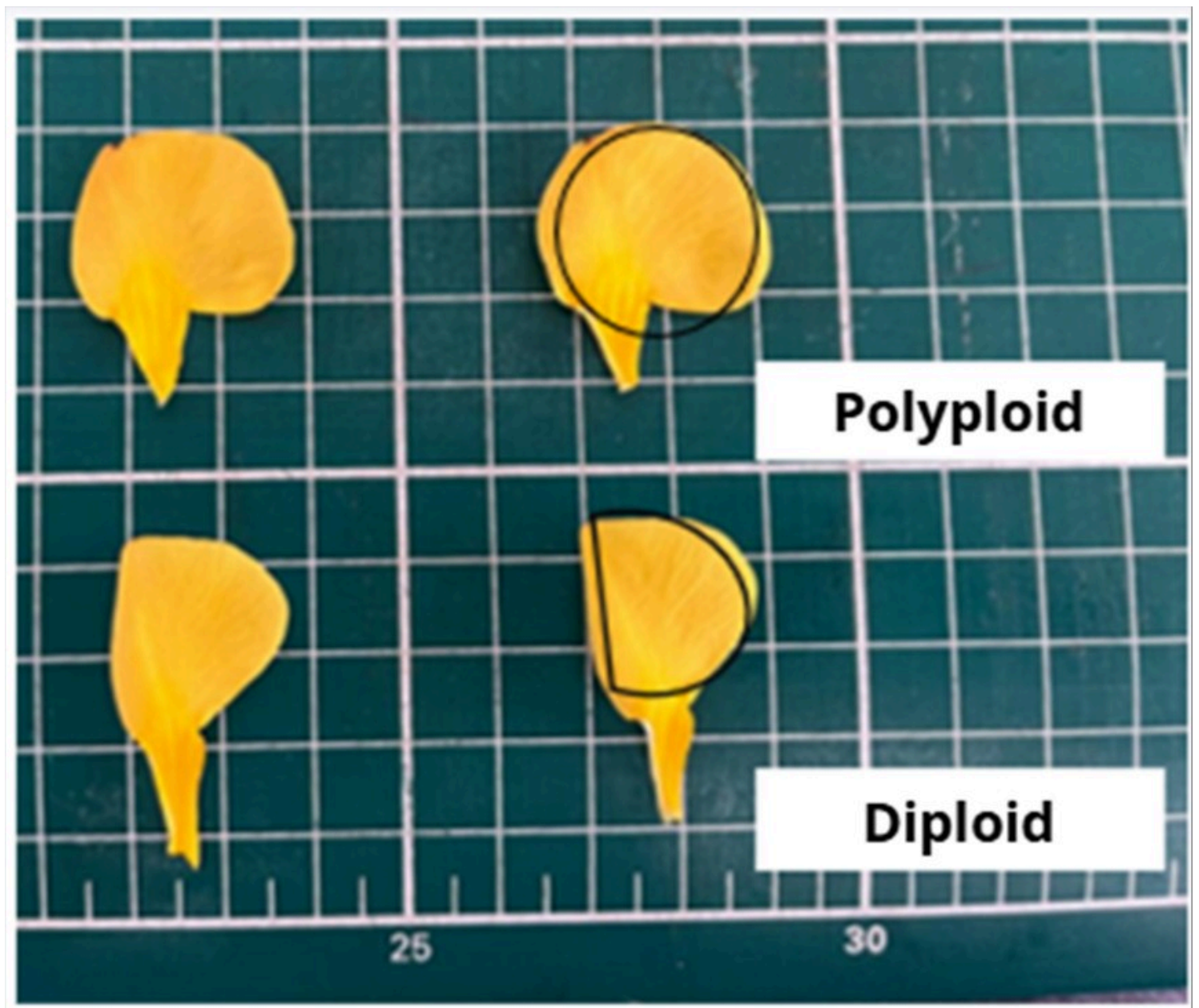


Figure 6

Petals of polyploid and diploid MD 1287 cultivar Variation in predominant shape in polyploid versus diploid plants

Supplementary Files

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