Eur. J. Hortic. Sci. *89*(6) | ISSN 1611-4426 print, 1611-4434 online | https://doi.org/10.17660/eJHS.2024/035 | © ISHS 2024

Original article

Polyploid induction of *Luculia pinceana* Hook. by aseptic germination combined with chemical agents

Lele Wang[1,2], Yefang Li[2], Xiaoyu Lu[2], Shifeng Li[3], Jie Song[3] and Wenling Guan[1,2]

[1] College of Resources and Environment, Yunnan Agricultural University, Kunming, China
[2] College of Horticulture and Landscape, Yunnan Agricultural University, Kunming, China
[3] Flower Research Institute, Yunnan Academy of Agricultural Sciences, Kunming, China

Summary

To obtain polyploid *Luculia pinceana* Hook., chemical mutagenesis, tissue culturing and rapid propagation technology were used, and the resulting germplasm may be bred to produce new varieties. The seeds of *L. pinceana* were used as induction materials, soaked in different oryzalin and colchicine concentrations, and then, mutant plants were obtained through seed germination. The variation rate of the plants was calculated on the basis of hypocotyl enlargement. Morphological characterization, stomatal characteristics, chromosome counts, and flow cytometry were used to identify mutant plants. The seeds soaked in 0.6% colchicine for 48 h had the best induction effect, and the mutation rate reached more than 70%. The chromosome count determined that the diploid chromosome number of *L. pinceana* was $2n = 2x = 44$ and the tetraploid chromosome number was $2n = 4x = 88$. In total, 11 tetraploid strains were identified by chromosome count and seven were identified by flow cytometry. Tetraploid plants had a dwarfed phenotype, with round leaves and thick roots, and a low density of large stomatal guard cells. In this study, a new polyploid *L. pinceana* germplasm was obtained for the first time, and it provided intermediate materials and a scientific basis for the breeding of new varieties.

Keywords
colchicine, *Luculia pinceana*, oryzalin, polyploid induction, variation identification

Significance of this study

What is already known on this subject?

- *Luculia pinceana* is a kind of wildflower resource with great ornamental value and development potential because of its many advantages, such as long flowering period, bright color, large and dense inflorescence, bright green leaves, and evergreen leaves. However, its low cold resistance and susceptibility to stem rot hinder the development and utilization of *L. pinceana* in gardens.

What are the new findings?

- The chromosome count determined that the diploid chromosome number of *L. pinceana* was $2n = 2x = 44$ and the tetraploid chromosome number was $2n = 4x = 88$. Tetraploid plants had a dwarfed phenotype, with round leaves and thick roots, and a low density of large stomatal guard cells.

What is the expected impact on horticulture?

- A new polyploid *L. pinceana* germplasm was obtained for the first time, and it provided intermediate materials and a scientific basis for the breeding of new varieties.

Introduction

Luculia pinceana is an evergreen shrub or tree belonging to the genus *Luculia* of the Rubiaceae family. It grows in west and northwest Guangxi, Guizhou, Yunnan, and Tibet. Native to forests or thickets on slopes, valleys, and streams at altitudes of 600–3,000 m, it is distributed throughout India, Nepal, Myanmar, and Vietnam (Ma et al., 2009; Tao and Taylor, 2011; Yang et al., 2010). *L. pinceana* is a kind of wildflower resource with great ornamental value and development potential because of its many advantages, such as long flowering period, bright color, large and dense inflorescence, bright green leaves, and evergreen leaves. However, its low cold resistance and susceptibility to stem rot hinder the development and utilization of *L. pinceana* in gardens. In addition, the selection of varieties is limited to natural variants, such as 'Hongfu' and 'Jinyudian', which have been developed in recent years (Li and Song, 2020; Liu et al., 2019; Ma et al., 2018).

Polyploid breeding is an important means of breeding new varieties of plants. Polyploid plants are often characterized by large flowers, strong stress resistance, robust growth, and large biomasses; consequently, polyploid methods have been widely used in plant breeding (Sattler et al., 2016). Octoploidy has been successfully induced by colchicine in *Neolamarckia cadamba* of the Rubiaceae family. Octoploid plants have thicker leaves, thicker midribs, lower stomatal densities, and larger stomatal sizes. This indicates that octoploid plants may respond well to environmental changes (Eng et al., 2021). However, polyploid breeding has not been reported in *Luculia*. In this study, a chemical mutagenesis method was used to obtain a new polyploid germplasm of *L. pinceana*, providing a germplasm material and a technical basis for further breeding of cold-resistant and disease-resistant new varieties of *L. pinceana*.

Polyploid induction is usually achieved by treating plant tissues with spindle inhibitors, such as colchicine, oryzalin, or trifluralin. Colchicine is the most commonly used chemical mutagen at present (Zhou et al., 2017). Colchicine disrupts spindle formation, causing chromosome doubling, and its concentration and processing time are interdependent. Depending on the method used, the concentration ranges from 0.05% to 1.0% and the treatment lasts from several

hours to several weeks. Higher concentrations and longer periods of colchicine treatment generally result in a higher explant mortality (Gantai et al., 2011). A higher concentration and longer treatment time for oryzalin also reduces the explant survival rate, but it increases the number of polyploids produced (Miguel and Leonhardt, 2011). Apical and lateral meristem, flower bud, root tip, and seed are usually used as materials for polyploid induction (Zakizadeh et al., 2020). Seed germination combined with an aseptic tissue-culture environment can minimize the influence of the environment on seed germination and seedling growth (Justamante et al., 2017), increase the reproduction rate, and save time and space (Kashtwari et al., 2021; Khan et al., 2011). The combination of polyploid induction and tissue culturing is easy to perform and the polyploids obtained are more stable (Eng and Ho, 2019).

In this study, the seeds of *L. pinceana* were used as the material, and colchicine and oryzalin were used independently as inducers. Then, the mutant plants were obtained by sterile tissue culture techniques, and identified by morphology, cytology, and flow cytometry. Combined, they provide a technical system for the polyploid breeding of *L. pinceana* that produced a new germplasm.

Materials and methods

Experimental materials and polyploid induction method

In October 2019, *L. pinceana* seeds were collected in Moduo County, Tibet Province, and stored in a refrigerator at 4°C. Full-grown seeds were screened before the experiment. The seeds were independently immersed in preparations of different concentrations of oryzalin (5 μmol L^{-1}, 15 μmol L^{-1}, and 25 μmol L^{-1}) and colchicine (0.2%, 0.6%, and 1%), for different times (oryzalin: 12 h, 24 h, and 48 h, and colchicine: 24 h, 48 h, and 72 h), with aseptic water as the blank control. The treated seeds were immersed in 5% sodium hypochlorite solution for 10 min, then washed three times with sterile water. The seeds were immersed in 75% alcohol solution for 2 min on the dissection table, washed three times with sterile water, and then inoculated onto MS medium. There were 10 seeds for each treatment group, and three replicates per group. The culture chamber temperature was 20° C ± 1°C with a 12-h light/12-h dark photoperiod.

Morphological identification and stomatal observation

Compared with the control group, hypocotyls appeared enlarged in the treated seeds after germination, and this was regarded as a characteristic of mutant plants. The germination and variation numbers of seeds were investigated, and the germination and variation rates of seeds were calculated. The germination rate = (number of germinated seeds/number of treated seeds) × 100% and the variation rate = (number of enlarged hypocotyls/number of treated seeds) × 100%.

After 4 months of growth, the tissue-cultured seedlings of *L. pinceana* were preliminarily identified, and leaf morphology and plant height were compared. The middle parts of mature leaves were selected, and the lower epidermis of each leaf was covered with transparent tape. Stomatal morphology was observed under an optical microscope (40×), and photos were taken using LAS EZ connected to the microscope (1,600 × 1,200 PX).

Chromosomal observations

The root tips of 1–2 cm of the same batch of tissue-cultured plantlets were used as materials. The chromosomes were prepared by pretreatment, fixation, dissociation, staining, and tablet pressing. The images were observed under an optical microscope and collected using LAS EZ. The ploidy of the 4-month-old plants were identified using the following method:

a) **Pretreatment:** pretreated with saturated p-dichlorobenzene at 20°C for 3 h;
b) **Fixation:** the root tip material was rinsed three times with distilled water and then placed in Carnoy's fixative solution (anhydrous ethanol:glacial acetic acid = 3:1, V/V) for 18 h at 4°C;
c) **Hydrolysis:** the root tip material was rinsed three times with distilled water and then was dissociated by acidolysis with 1 mol L^{-1} hydrochloric acid solution at 60°C for 10 min.
d) **Staining and tablet pressing:** the separated root tips were rinsed three times with distilled water and then soaked in modified phenol fuchsin staining solution for 30 min. Then, the root tip material was placed on a slide to isolate the meristem area of the root tip, covered with a cover glass, and gently knocked vertically with an eraser at a uniform speed.

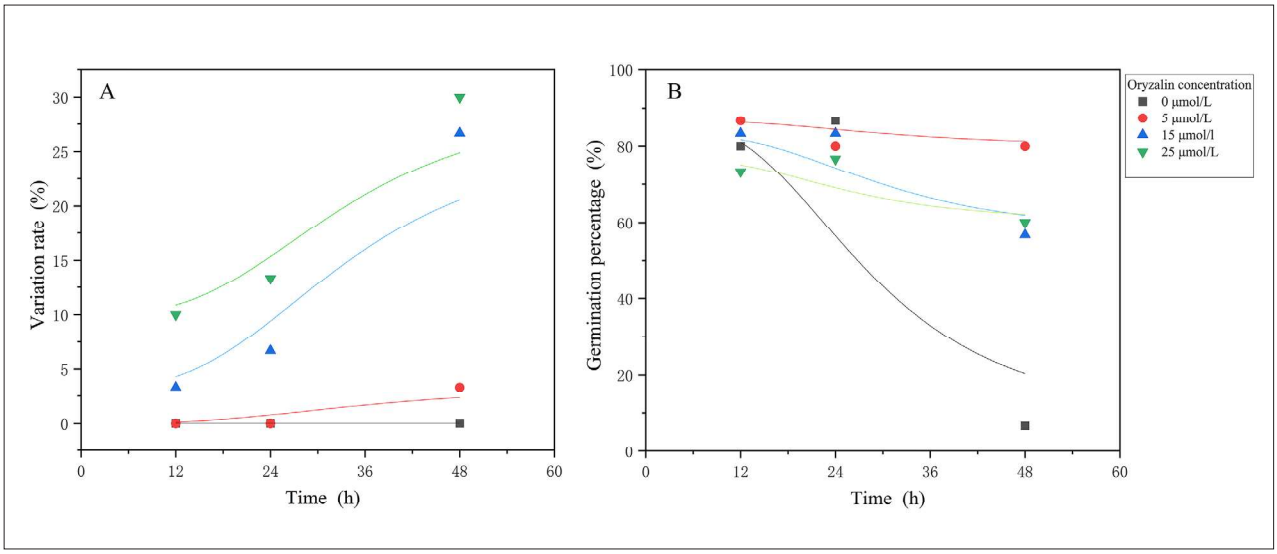


Figure 1. Effects of oryzalin concentration on variation rate (A) and germination rate (B) of *L. pinceana* seeds.

Table 1. Effects of oryzalin on *L. pinceana* seed germination and variation.

Oryzalin concentration (μmol L^{-1})	Duration of treatment (h)	Number of seeds	Germination percentage (%)	Variation rate (%)
0	12	30	80.00abc	0.00c
0	24	30	86.67a	0.00c
0	48	30	86.67a	0.00c
5	12	30	86.67a	0.00c
5	24	30	80.00abc	0.00c
5	48	30	80.00abc	3.33bc
15	12	30	83.33abc	3.33bc
15	24	30	83.33ab	6.67bc
15	48	30	56.67c	26.67a
25	12	30	73.33abc	10.00bc
25	24	30	76.67abc	13.33b
25	48	30	60.00bc	30.00a

Different lowercase letters in the same column indicate significant differences among treatments at the 0.05 level ($P<0.05$) among treatment by LSD test.

e) **Microscopic observation and image collection:** in total, selecting 30 pictures of chromosomes from each of the different ploidy plants. Among them, more than 85% of the cells had the same chromosome number, which was consequently considered the chromosome number of the plant (Li and Chen, 1985).

Flow cytometry

Tomato and corn A188 were used as internal references, and the suspension of the sample to be tested was mixed with the suspension of the internal reference sample in an appropriate proportion. BD FACScalibur flow cytometry was used to detect the nuclear suspension samples after staining. Blue light excitation at 488 nm was used to detect the emission light fluorescence intensity of propidium iodide. In total, 10,000 particles were collected each time. The coefficient of variation was controlled within 5%. Modifit3.0 analysis software was used to map and analyze the data (Doležel et al., 2007).

Data analysis

All data were sorted out by Excel 2020. SPSS 17.0 was used for one-way analysis of variance, and least-significant difference (LSD) tests. Figures were plotted using Origin 2018.

Results

Effects of oryzalin concentration on seed germination of *L. pinceana*

As the oryzalin concentration and induction time increased, the germination rate decreased and the mutation rate increased (Figure 1). The germination rates of *L. pinceana* seeds soaked in 15 μmol L^{-1} and 25 μmol L^{-1} oryzalin for 48 h were significantly different from that of the control (0 μmol L^{-1}), but there were no significant differences in other concentrations and induction times. Compared with the control, the mutation rate at 25 μmol L^{-1} and 48 h increased to 30%. In conclusion, the optimal treatment was to soak the seeds in 25 μmol L^{-1} oryzalin for 48 h (Table 1).

Effects of colchicine treatments on *L. pinceana* seeds

As the colchicine concentration and treatment time increased, the germination rate of seeds decreased and the mutation rate increased (Figure 2). The germination rates of seeds soaked in 0.2%, 0.6%, and 1% colchicine for 72 h were significantly different from that of the control. The mutation rate at 0.6% colchicine and 48 h reached to 70%. The induction effect was the best, and the germination rate was the

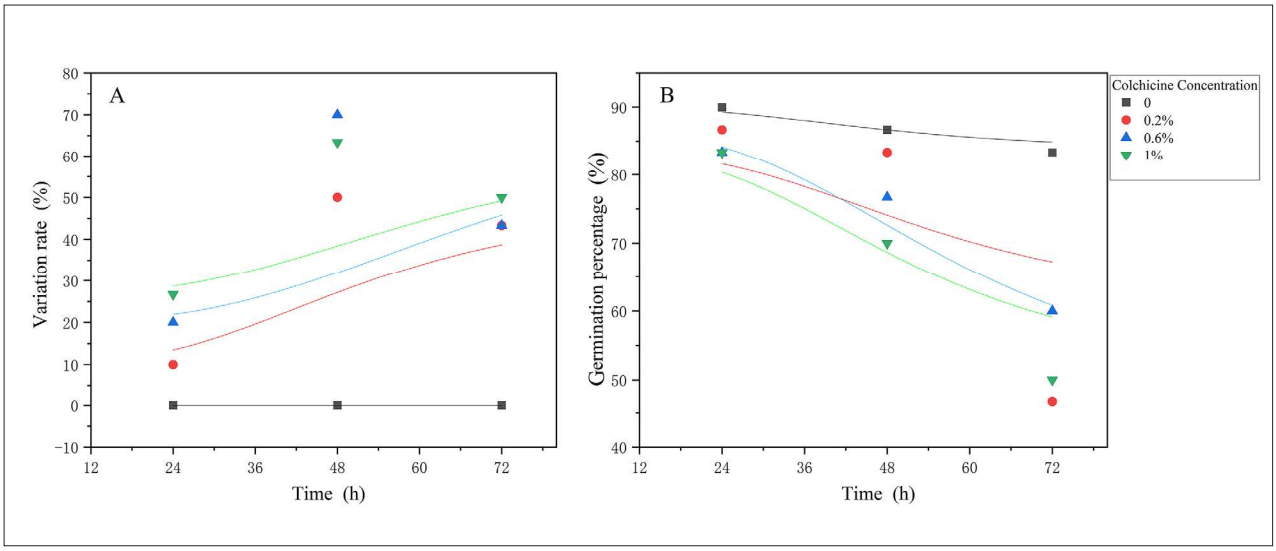


Figure 2. Effects of colchicine concentration on variation rate (A) and germination rate (B) of *L. pinceana* seeds.

Table 2. Effects of colchicine on *L. pinceana* seed germination and variation.

Colchicine concentration (%)	Duration of treatment (h)	Number of seeds	Germination percentage (%)	Variation rate (%)
0	24	30	90.00a	0.00f
0	48	30	86.67a	0.00f
0	72	30	83.33abc	0.00f
0.2	24	30	86.67ab	10.00ef
0.2	48	30	83.33abc	50.00bc
0.2	72	30	46.67d	43.33cd
0.6	24	30	83.33abc	20.00e
0.6	48	30	76.67abc	70.00a
0.6	72	30	60.00cd	43.33cd
1	24	30	83.33ab	26.67de
1	48	30	70.00bcd	63.33ab
1	72	30	50.00d	50.00bc

Different lowercase letters in the same column indicate significant differences among treatments at the 0.05 level ($P<0.05$) among treatment by LSD test.

highest among the treatments. Therefore, the best treatment was to soak the seeds with 0.6% colchicine for 48 h (Table 2).

Observations of *L. pinceana* morphology and stomata

Hypocotyl enlargement was observed in the seedlings treated with chemical inducers after germination. Compared with the diploid plants, it was found that the mutant plants were shorter. The diploid leaves were long and narrow, whereas the leaves of the mutant were more round and thicker, darker, and slightly curly (Figure 3). The stomatal guard cells of the mutant had larger volumes but were less in density than those of the diploid plant (Figure 4).

Identification of chromosomes of mutant plants

Chromosome identification was performed on 12 morphologically identified mutant strains, and 11 plants were tetraploid (Table 3). The mutation identified by morphological observation had a high probability of being identified as tetraploid on the basis of its chromosome number, which further indicated that the morphological identification was reliable.

In total, 26 chromosome images of diploid plants showed 44 chromosomes, accounting for 86.67% (>85%) of the total number of images; consequently, $2n = 2x = 44$ (Figure 5A). In total, 27 images of tetraploid plants showed 88 chromosomes, accounting for 90% (>85%) of the total number of images; consequently, $4n = 4x = 88$ (Figure 5B).

Flow cytometry

Flow cytometry was used to confirm the 8 morphologically identified mutant strains (Table 4). For diploid plants, G = 0.80 ± 0.17 pg, whereas for tetraploid plants, G =

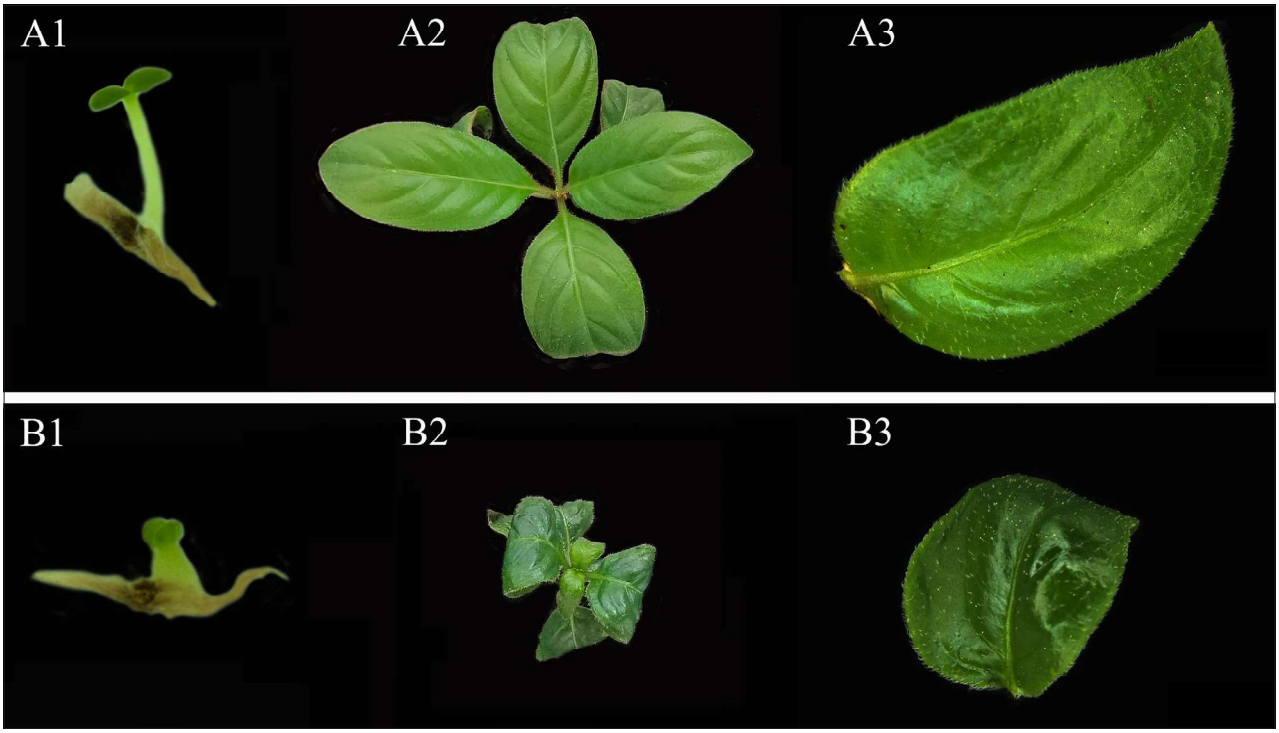


Figure 3. Morphological observation on diploid and variant strains of *L. pinceana*. Cotyledon stage of control plant (A1); Control plant seedling stage (A2); Leaf of control plant (A3); Cotyledon stage of mutant (hypocotyl enlarged) (B1); Mutant seedling stage (Leaf curl) (B2); Mutant leaf (leaves shrivel) (B3).

Table 3. Chromosome count identifies the number of polyploidy strains.

Inducer	Concentration	Induction time (h)	Number of identified plants	Number of tetraploid plants
Oryzalin	15 μmol L^{-1}	48	3	2
Oryzalin	25 μmol L^{-1}	48	3	3
Colchicine	0.6%	48	3	3
Colchicine	1%	48	3	3

1.59 ± 0.21 pg. Thus, the DNA contents of the different ploidy plants had an approximately multiple relationship (Figure 6). A constructed histogram revealed two obvious peaks in the control plant, corresponding to the fluorescence intensity of internal reference and that of the sample. The average peak position of the tetraploid plants was twice that of diploid plants.

Discussion

Polyploid germplasm of *L. pinceana*

Luculia pinceana has a high ornamental value and many application prospects, but it has the disadvantages of poor cold resistance and susceptibility to stem rot. Enhancing resistance is the main *L. pinceana* breeding goal. Polyploid plants have increased soluble sugar and enzyme contents due to chromosome doubling, which improves stress resistance. For example, the physiological index activity of stress resistance in a polyploid plant is significantly different from that in a control diploid plant, and the stress resistance of the former is stronger (Yue et al., 2020). Chromosome doubling can also lead to resistance against environmental stresses, such as drought, salt, cold and high temperature (Del Pozo and Ramirez-Parra, 2015). In this study, the innovative technology of polyploid germplasm of *L. pinceana* was preliminarily investigated, and 11 *L. pinceana* variant strains with chromosome doubling were obtained. The chromosomes of *L. pinceana*, $2n = 2x = 44$ and $2n = 4x = 88$ were obtained by

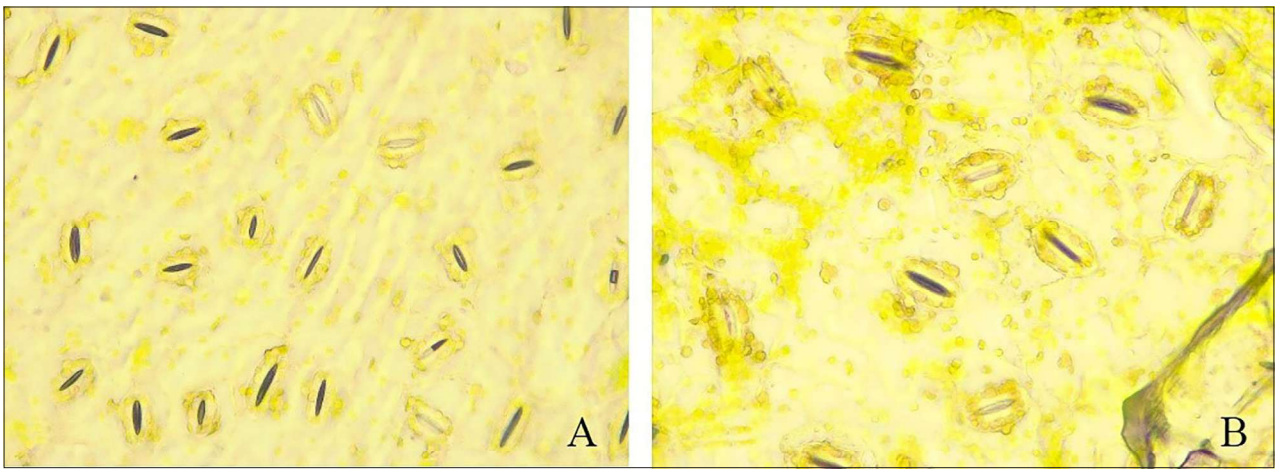


Figure 4. Observation of leaf stomatal morphology of diploid and variant strains of *L. pinceana*. Size and density of stomata of diploid (A); size and density of stomata of variant strains (B).

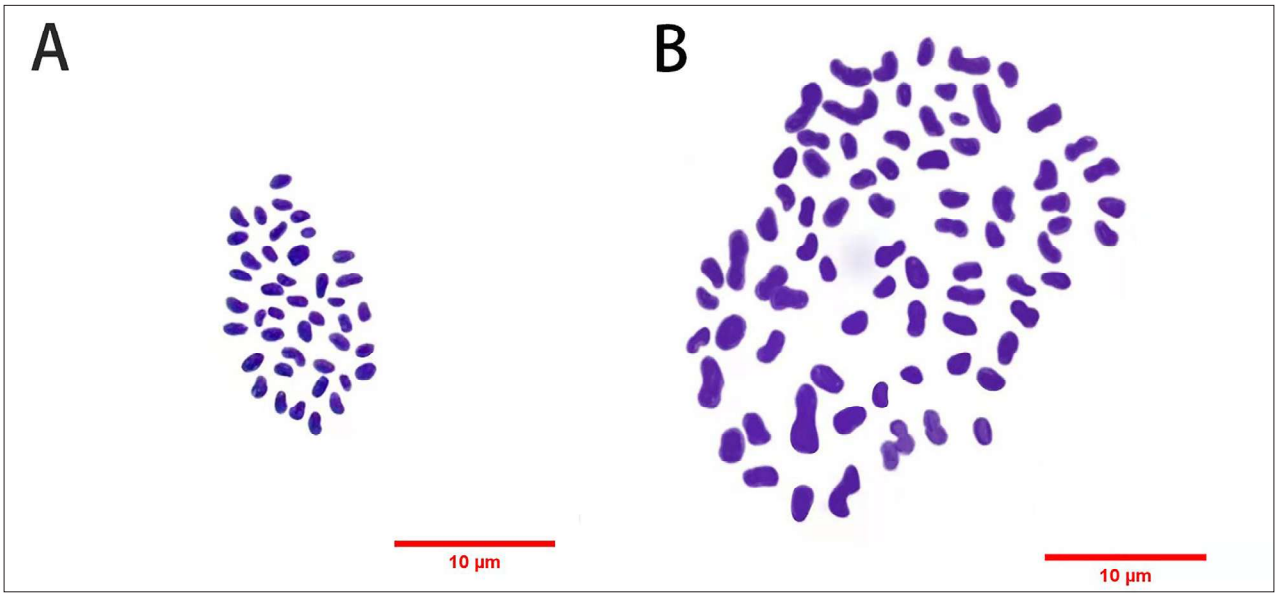


Figure 5. Chromosome identification of diploid and tetraploid *L. pinceana*. Diploid plant 100 × whole-cell image under the objective lens (A); enlarged chromosome image of tetraploid plant 100 × objective lens (B).

Table 4. Flow cytometry identifies the number of polyploidy strains.

Inducer	Concentration	Induction time (h)	Serial number	Number of identified plants	Number of tetraploid plants
Colchicine	0.2%	48	A1-5	5	4
Colchicine	0.6%	48	B	1	0
Colchicine	1%	48	C1	1	1
Colchicine	1%	72	C2	1	1

chromosome preparation, and the chromosome of *Luculia gratissima*, $2n = 2x = 44$, was obtained by Selvaraj (1987), and it was consistent with our results. During polyploid induction, chimeras and mutants may be produced. The initial in vivo application of colchicine to seedling apical tips produced mixoploid plants of petunia at a high rate of efficiency (Ning et al., 2009). Tetraploid and mixoploid *Limonium sinuatum* plants were successfully obtained with oryzalin treatment (Mori et al., 2021). But Glowacka et al. (2010) found that in vitro colchicine treatment during callus induction and during shoot regeneration from callus resulted in no chimeric polyploids as well as a very low number of albinos (2.5%). In this study, colchicine and oryzalin treatment resulted in no chimeric polyploids. It is possible that we did not identify all induced plants, but selected plants with morphological variation to be identified by chromosome identification and flow cytometry. Thus, it was feasible to induce polyploidy in *L. pinceana* germplasm, and new varieties may be produced through polyploid induction. In the future, the differences in the resistance index between polyploid and diploid plants should be studied to provide a scientific basis for the breeding of resistant polyploid varieties.

Selection of polyploid inducible materials

Chemical treatments to induce polyploid are effective methods to increase the mutagenesis rate and obtain polyploids in ornamental plants (Manzoor et al., 2019; Sattler et al., 2016). Polyploid induction theory indicates that cells with strong division capabilities are more likely to be induced polyploids. Therefore, it is necessary to select tissues or organs with strong cell division and proliferation capabilities as induction materials. In previous studies, seeds, adventitious buds, and shoot tips have been commonly used as materials for polyploid induction (Eng and Ho, 2019; He et al., 2016). Hwang et al. (2015) found in polyploid induction of *Cymbidium Hybrids*, colchicine was more efficient than oryzalin in terms of polyploidy induction. Pickens et al. (2006) found that the colchicine is less toxic on poinsettia tissues and shoot induction than oryzalin in polyploid induction of *Euphorbia pulchurrima* 'Winter Rose'. In this study, two inducers, oryzalin, and colchicine, were used to induce polyploidy in *L. pinceana* seeds. From the perspective of chemical inducer effect and seed effect, colchicine was better as an inducer. Chu et al. (2014) used three kinds of inducers to explore the induction of *Anthurium andraeanum* polyploidy. The oryzalin induction method effectively reduces mortality, but the induction effect is not as good as those of colchicine and trifluralin. However, the chromosomal destruction of colchicine leads to more chimerism, and the induction effect needs to be considered comprehensively. Švécarová et al. (2019) induced young stem segments of *Humulus lupulus* L. by oryzalin and obtained tetraploid plants without chimeras. In this study, only seeds were used as induction materials. Buds, shoot tips, calli, and other tissues could be used as experimental materials in the future. The use of aseptic tissue culturing made it easier for the induced seedlings to survive, and it facilitated the separation and stability of chimeras, as well as the propagation of mutant strains.

Ornamental properties of polyploid plants

Artificially polyploid-induction techniques have been used to improve flower morphology, fruit size, and yield, as well as to break the self-incompatibility system, restore fertility, and improve pest and disease resistance (Tel-Zur

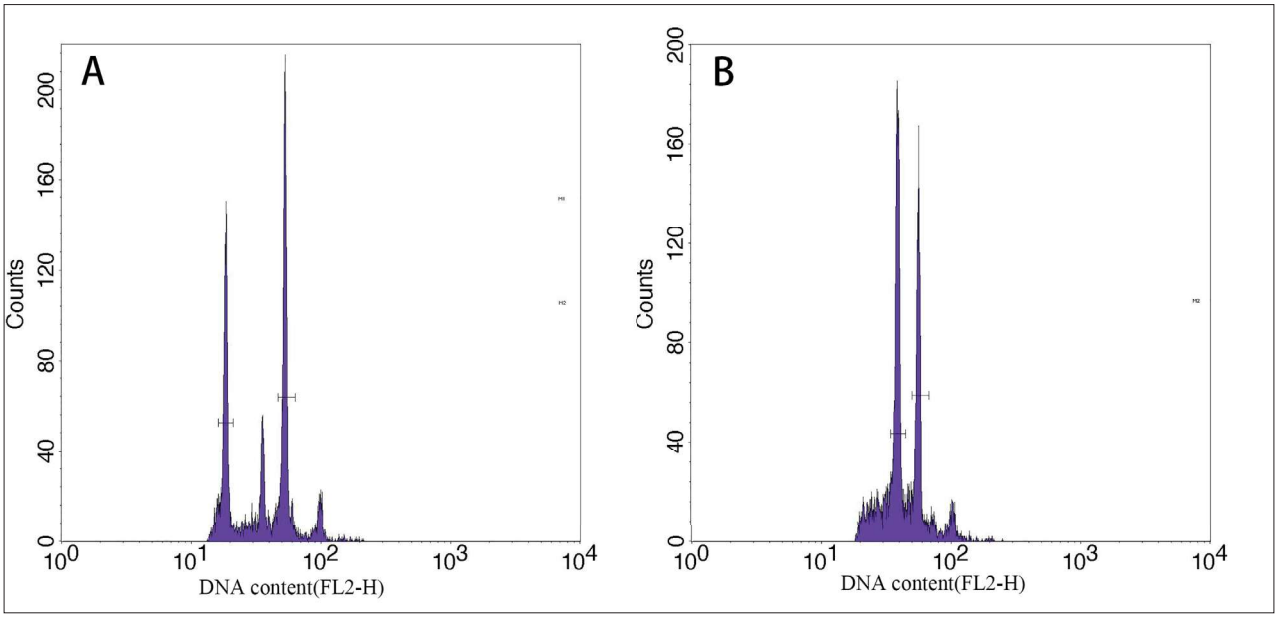


Figure 6. Flow cytometry identification of diploid and tetraploid *L. pinceana*. FCM histogram for diploid plants (A); FCM histogram for tetraploid plants (B).

et al., 2011; Ye et al., 2010). Polyploid orchids have the ideal characteristics of larger flowers, richer materials, rounder structures, and darker colors compared with non-polyploid orchids (Miguel and Leonhardt, 2011). Tetraploid plants have larger flowers, longer stems, and longer vase life than diploid plants, and this enhances *Gerbera jamesonii*'s ornamental value (Gantait et al., 2011). For ornamental purposes, the use of colchicine-induced thick stem wings and inflorescence clogs in the tetraploid *Limonium sinuatum* (L.) are undesirable, resulting in the tetraploid being of low ornamental value (Mori et al., 2021). In most tetraploid *Lepidium sativum* L., the first leaf at the seedling stage is distorted but returns to normal as the plant grows (Aqafarini et al., 2019). In this study, the induced tetraploid plants had slightly deformed and curly leaves at the early growth stage, which is different from the general characteristics of polyploid plants, but this might be related to the growth stage. However, the leaves were rounder, thicker, and darker than those of the diploids. Therefore, the ornamental value of tetraploid *L. pinceana* requires further observation.

Effect of inducer concentration on seed germination

Colchicine and sulfamide have inhibitory effects on plant development (Li and Ruter, 2017). The treatment times and concentrations of chemical inducers are critical for polyploid induction (Elyazid and El-shereif, 2014). For *Eriobotrya japonica* seeds treated with 0.5% colchicine for 48 h showed a higher mortality but produced more stable tetraploid plants (Blasco et al., 2015). Rubuluza et al. (2007) treated *Colophospermum mopane* seeds with 0.1% colchicine for 48 h and found that the polyploid-induced mutation rate was higher, but it was harmful to seed development and was not conducive to growth and survival. Azalea seeds treated with 30 μmol L^{-1} oryzalin for 16 h produce more tetraploids and octoploids, but the seed germination rate decreases (Mo et al., 2020). In this study, the germination rate of seeds decreased as the colchicine and oryzalin concentrations increased. The variation rates for the 25-μmol L^{-1} oryzalin treatment for 48 h and the 0.6%-colchicine treatment for 48 h were the highest, but the germination rates were low. These results were consistent with a high concentration of inducer inhibiting seed germination but inducing a higher rate of polyploid variation. The inhibitory effects of a high inducer concentration and treatment time on seed germination may be related to the infiltration of compounds through seed coats and the bioactive substances in seeds (Blasco et al., 2015; Yan et al., 2022).

Polyploid identification method

Polyploid identification methods can be divided into indirect and direct. Most polyploid studies use the former first and then the latter to accurately identify polyploids (Eng and Ho, 2019). The indirect identification of polyploid leaves using morphological and anatomical characteristics is common. Chen et al. (2011) found that polyploid *A. andraeanum* leaves are thicker and darker than diploid leaves. Bhattarai et al. (2021) induced polyploid *G. jamesonii* that had wider and thicker leaves than the diploid. Most polyploidy studies show similar stomatal characteristics, such as stomatal size, stomatal density, guard cell size, and guard cell chloroplast number in polyploids (Xie et al., 2015). In this study, the tetraploid *L. pinceana* leaves were thicker, darker, and more rounded than those of diploids, and the stomata were larger and less dense. However, morphological methods may not be accurate, and some morphological and anatomical features may be altered by environmental factors. The identification and counting of polyploid chromosome numbers is a direct, unambiguous, and standard method for determining the number of chromosomes using microscopic observations and flow cytometry (Kim et al., 2019). Flow cytometry is an important and convenient method to measure genome size and evaluate the success rate of polyploid induction (Doležel et al., 2012).

Conclusions

Here, the seeds of *L. pinceana* were treated with colchicine or oryzalin, and then, the mutant plants were obtained by aseptic culturing. The best treatment for polyploid induction was 0.6% colchicine for 48 h, with 70% of mutation rate and a 76% germination rate. The chromosome number of the diploid was $2n = 2x = 44$, and that of the tetraploid was $2n = 4x = 88$. In total, 11 tetraploid strains were identified by chromosome counts and seven were identified by flow cytometry. Compared with diploid plants, tetraploid plants were shorter, with rounder, darker leaves, and larger, but less dense, stomatal guard cells. The results of this study provide an important technical basis, as well as a new germplasm, for the breeding of *L. pinceana*, and they have important significance for the horticultural development of new *L. pinceana* varieties.

Acknowledgments

The research was supported by the Project of Scientific Research Fund of Yunnan Education Department (2021J0137), "Study on induction of polyploidy in characteristic flower *Luculia pinceana* Hook.".

Conflict of interest

The authors declare that they have no conflict of interest.

References

Aqafarini, A., Lotfi, M., Norouzi, M., and Karimzadeh, G. (2019). Induction of tetraploidy in garden cress: Morphological and cytological changes. Plant Cell. Tissue Organ Cult. *137*, 627–635. https://doi.org/10.1007/s11240-019-01596-5.

Bhattarai, K., Kareem, A., and Deng, Z. (2021). *In vivo* induction and characterization of polyploids in gerbera daisy. Sci. Hortic. (Amsterdam) *282*, 110054. https://doi.org/10.1016/j.scienta.2021.110054.

Blasco, M., Badenes, M.L., and Naval, M. del M. (2015). Colchicine-induced polyploidy in loquat (*Eriobotrya japonica* (Thunb.) Lindl.). Plant Cell. Tissue Organ Cult. *120*, 453–461. https://doi.org/10.1007/s11240-014-0612-3.

Chen, C., Hou, X., Zhang, H., Wang, G., and Tian, L. (2011). Induction of *Anthurium andraeanum* 'Arizona' tetraploid by colchicine *in vitro*. Euphytica *181*, 137–145. https://doi.org/10.1007/s10681-010-0344-3.

Chu, L.H., Peng, J.J., Wang, Z., Zhao, K., Yuan, S.X., Ming, J., and Liu, C. (2014). Studies on polyploid induction in *Anthurium andraeanum* using oryzalin, trifluralin and colchicine. Acta Hortic. Sinica *6*. https://doi.org/10.16420/j.issn.0513-353x.2014.11.015.

Del Pozo, J.C., and Ramirez-Parra, E. (2015). Whole genome duplications in plants: An overview from *Arabidopsis*. J. Experim. Bot. *66*, 6991–7003. https://doi.org/10.1093/jxb/erv432.

Doležel, J., Greilhuber, J., and Suda, J. (2007). Flow cytometry with plants: An overview. In Flow Cytometry with Plant Cells: Analysis of Genes, Chromosomes and Genomes, J. Doležel, J. Greilhuber, and J. Suda, eds. (Wiley), p. 41–66. https://doi.org/10.1002/9783527610921.ch3.

Doležel, J., Vrána, J., Šafář, J., Bartoš, J., Kubaláková, M., and Šimková, H. (2012). Chromosomes in the flow to simplify genome analysis. Funct. Integr. Genomics *12*, 397–416. https://doi.org/10.1007/s10142-012-0293-0.

Elyazid, D.M.A., and El-shereif, A.R. (2014). *In vitro* induction of polyploidy in *Citrus reticulata* Blanco. Am. J. Plant Sci. *5*, 1679–1685.

Eng, W.H., and Ho, W.S. (2019). Polyploidization using colchicine in horticultural plants: A review. Sci. Hortic. (Amsterdam) *246*, 604–617. https://doi.org/10.1016/j.scienta.2018.11.010.

Eng, W.H., Ho, W.S., and Ling, K.H. (2021). *In vitro* induction and identification of polyploid *Neolamarckia cadamba* plants by colchicine treatment. PeerJ *9*. https://doi.org/10.7717/peerj.12399.

Gantait, S., Mandal, N., Bhattacharyya, S., and Das, P.K. (2011). Induction and identification of tetraploids using *in vitro* colchicine treatment of *Gerbera jamesonii* Bolus cv. Sciella. Plant Cell. Tissue Organ Cult. *106*, 485–493. https://doi.org/10.1007/s11240-011-9947-1.

Glowacka, K., Jeżowski, S., and Kaczmarek, Z. (2010). Impact of colchicine application during callus induction and shoot regeneration on micropropagation and polyploidisation rates in two *Miscanthus* species. In Vitro Cell. Developm. Biol. – Plant *46*, 161–171.

He, M., Gao, W., Gao, Y., Liu, Y., Yang, X., Jiao, H., and Zhou, Y. (2016). Polyploidy induced by colchicine in *Dendranthema indicum* var. *aromaticum*, A scented chrysanthemum. Eur. J. Hortic. Sci. *81*, 219–226. https://doi.org/10.17660/eJHS.2016/81.4.5.

Hwang, S.H., Kim, M.S., and Park, S.Y. (2015). Improvement of chromosome doubling efficiency in *Cymbidium* hybrids by colchicine and oryzalin treatment. Hortic. Sci. Technol. *33*(6), 900–910. https://doi.org/10.7235/hort.2015.15063.

Justamante, M.S., Ibáñez, S., Villanova, J., and Pérez-Pérez, J.M. (2017). Vegetative propagation of argan tree (*Argania spinosa* (L.) Skeels) using *in vitro* germinated seeds and stem cuttings. Sci. Hortic. (Amsterdam) *225*, 81–87. https://doi.org/10.1016/j.scienta.2017.06.066.

Kashtwari, M., Jan, S., Wani, A.A., and Dhar, M.K. (2021). Induction of polyploidy in saffron (*Crocus sativus* L.) using colchicine. J. Crop Improv. *36*(4), 555–581. https://doi.org/10.1080/15427528.2021.1994502.

Khan, M.A., Nagoo, S., Naseer, S., Nehvi, F.A., and Zargar, S.M. (2011). Induced mutation as a tool for improving corm multiplication in Saffron (*Crocus sativus* L.). J. Phytol. *3*, 8–10.

Kim, J.S., Seo, M.-S., Moon, M.-S., Won, S.Y., and Kwon, S.-J. (2019). Insight on doubled haploid production with an amphidiploid species 'Dolsangat' in *Brassica juncea*. Korean J. Breed. Sci. *51*, 341–350. https://doi.org/10.9787/kjbs.2019.51.4.341.

Li, M.X., and Chen, R.Y. (1985). Standardization of plant karyotype analysis. J. Wuhan Bot. Res. 297–302.

Li, S.F., and Song, J. (2020). Characteristic wild flowers – *Luculia pinciana*. China Flowers Hortic. 1.

Li, Z., and Ruter, J.M. (2017). Development and evaluation of diploid and polyploid *Hibiscus moscheutos*. HortScience *52*, 676–681. https://doi.org/10.21273/HORTSCI11630-16.

Liu, X.X., Ma, H., Wan, Y.M., Zhao, Z.G., and Li, Z.H. (2019). A new *Luculia pinceana* cultivar 'Jinyudian.' Acta Hortic. Sinica *46*, 199–200.

Ma, H., Wang, Y., and Liang, N. (2009). A study on the breeding system of *Luculia pinciana*. For. Res. *22*, 6.

Ma, H., Wan, Y.M., Liu, X.X., and Zhao, Z.G. (2018). 'Hongfu', a new red-flowered *Luculia pinceana* cultivar. Acta Hortic. Sinica *45*(10), 2067–2068. https://doi.org/10.16420/j.issn.0513-353x.2018-0065.

Manzoor, A., Ahmad, T., Bashir, M.A., Hafiz, I.A., and Silvestri, C. (2019). Studies on colchicine induced chromosome doubling for enhancement of quality traits in ornamental plants. Plants *8*, 1–16. https://doi.org/10.3390/plants8070194.

Miguel, T.P., and Leonhardt, K.W. (2011). *In vitro* polyploid induction of orchids using oryzalin. Sci. Hortic. (Amsterdam) *130*, 314–319. https://doi.org/10.1016/j.scienta.2011.07.002.

Mo, L., Chen, J.H., Chen, F., Xu, Q.W., Tong, Z.K., Huang, H.H., Dong, R.H., Lou, X.Z., and Lin, E.P. (2020). Induction and characterization of polyploids from seeds of *Rhododendron fortunei* Lindl. J. Integr. Agric. *19*, 2016–2026. https://doi.org/10.1016/S2095-3119(20)63210-5.

Mori, S., Yahata, M., Kuwahara, A., Shirono, Y., Ueno, Y., Hatanaka, M., Honda, Y., Sugiyama, K., Murata, N., Okamoto, Y., and Wagatsuma, T. (2021). Morphological characterization of tetraploids of *Limonium sinuatum* (L.) Mill. produced by oryzalin treatment of seeds. Horticulturae *7*, 1–10. https://doi.org/10.3390/horticulturae7080248.

Ning, G.G., Shi, X.P., Hu, H.R., Yan, Y., and Bao, M.Z. (2009). Development of a range of polyploid lines in *Petunia hybrida* and the relationship of ploidy with the single-/double-flower trait. HortScience *44*(2), 250–255. https://doi.org/10.21273/HORTSCI.44.2.250.

Pickens, K.A., Kania, S.A., and Cheng, Z.M. (2006). Effects of colchicine and oryzalin on callus and adventitious shoot formation of *Euphorbia pulchurrima* 'Winter Rose'. HortScience *41*(7), 1651–1655.

Rubuluza, T., Nikolova, R.V., Smith, M.T., and Hannweg, K. (2007). *In vitro* induction of tetraploids in *Colophospermum mopane* by colchicine. South African J. Bot. *73*, 259–261. https://doi.org/10.1016/j.sajb.2006.12.001.

Sattler, M.C., Carvalho, C.R., and Clarindo, W.R. (2016). The polyploidy and its key role in plant breeding. Planta *243*, 281–296. https://doi.org/10.1007/s00425-015-2450-x.

Selvaraj, R. (1987). Karyomorphological studies in south Indian Rubiaceae. Cytologia (Tokyo) *52*, 343–356. https://doi.org/10.1508/cytologia.52.343.

Švécarová, M., Navrátilová, B., Hašler, P., and Ondřej, V. (2019). Artificial induction of tetraploidy in *Humulus lupulus* L. using oryzalin. Acta Agrobot. *72*, 1–10. https://doi.org/10.5586/aa.1764.

Tao, C., and Taylor, C.M. (2011). *Luculia*. Flora of China *19*, 214–215.

Tel-Zur, N., Dudai, M., Raveh, E., and Mizrahi, Y. (2011). *In situ* induction of chromosome doubling in vine cacti (*Cactaceae*). Sci. Hortic. (Amsterdam) *129*, 570–576. https://doi.org/10.1016/j.scienta.2011.04.027.

Xie, X., Agüero, C.B., Wang, Y., and Walker, M.A. (2015). *In vitro* induction of tetraploids in *Vitis* × *Muscadinia* hybrids. Plant Cell. Tissue Organ Cult. *122*, 675–683. https://doi.org/10.1007/s11240-015-0801-8.

Yan, Y.J., Qin, S.S., Zhou, N.Z., Xie, Y., and He, Y. (2022). Effects of colchicine on polyploidy induction of *Buddleja lindleyana* seeds. Plant Cell. Tissue Organ Cult. *149*, 735–745. https://doi.org/10.1007/s11240-022-02245-0.

Yang, X.Q., Wang, C.X., Tong, Y.L., and Xiong, L. (2010). A fast intelligent non-tube seeding cultivation for *Luculia* intemedia. J. West China For. Sci. *39*, 69–73.

Ye, Y.M., Tong, J., Shi, X.P., Yuan, W., and Li, G.R. (2010). Morphological and cytological studies of diploid and colchicine-induced tetraploid lines of crape myrtle (*Lagerstroemia indica* L.). Sci. Hortic. (Amsterdam) *124*, 95–101. https://doi.org/10.1016/j.scienta.2009.12.016.

Yue, M., Yang, S.G., Chen, J., Zhang, J.G., and Guo, C.M. (2020). Study on physiological indexes of polyploid resistance of *Scutellaria*

barbata D.Don. Mod. Hortic. *43*, 3. https://doi.org/10.14051/j.cnki.xdyy.2020.19.008.

Zakizadeh, S., Kaviani, B., and Hashemabadi, D. (2020). In vivo-induced polyploidy in *Dendrobium* 'Sonia' in a bubble bioreactor system using colchicine and oryzalin. Rev. Bras. Bot. *43*, 921–932. https://doi.org/10.1007/s40415-020-00654-z.

Zhou, H.W., Zeng, W.D., and Yan, H.B. (2017). *In vitro* induction of tetraploids in cassava variety 'Xinxuan 048' using colchicine. Plant Cell. Tissue Organ Cult. *128*, 723–729. https://doi.org/10.1007/s11240-016-1141-z.

Received: Jul. 19, 2023
Accepted: Mar. 11, 2024

Addresses of authors:
Lele Wang[1,2], Yefang Li[2], Xiaoyu Lu[2], Shifeng Li[3], Jie Song[3] and Wenling Guan[1,2,*]
[1] College of Resources and Environment, Yunnan Agricultural University, Kunming, China
[2] College of Horticulture and Landscape, Yunnan Agricultural University, Kunming, China
[3] Flower Research Institute, Yunnan Academy of Agricultural Sciences, Kunming, China
* Corresponding author