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Effect of Gout Drug Treatments on Survival Rate and Morphological Change of *Lindernia* sp. *in vitro*

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ABSTRACT

This research created greater genetic variation in *Lindernia* sp. by studying the most appropriate concentration and duration of colchicine treatment that could induce polyploidy in *Lindernia* sp. Leaf and node sections taken from 1-month old Lindernia plants grown *in vitro* were used as the explants to be immersed in colchicine solution at the concentrations of 0, 5, 10, 15 and 20 ppm for 1, 2 and 3 days. The experiment was a 5×3 Factorial in CRD with 10 replications and 2 explants per replication. The results showed that the plants regenerated from the colchicine-treated node and leaf sections had lower survival rate, shorter plant height, shorter internode length, fewer roots and shorter roots than the control group, with an increasing effect from increased concentrations of colchicine and increased exposure time; but the colchicine-treated plants had thicker stems, thicker roots and wider and longer leaves than the control. The treatment group exposed to colchicine at the concentration of 10 ppm for 3 days had the lowest survival rate (60% for node sections and 50% for leaf sections), but the highest polyploidy induction rate at 22.00% for node sections and 16.22% for leaf sections. The frequency of polyploidy induction was higher in node sections than in leaf sections, and the plants regenerated from node sections were also stronger and had a higher survival rate when planted out than those regenerated from leaf sections.

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1. Introduction

Lindernia sp. is a creeping herbaceous plant in the family Linderniaceae with a mostly procumbent habit reaching a height of 5 – 20 cm. The stems are angular and the leaves single and opposite with leaf margins sinuate, serrate, or entire. The flowers are small, purple and bilabiate in form with a long fused corolla tube (Smitinand, 1990). *Lindernia* sp. can tolerate a wide range of environmental conditions as they are found growing naturally in many areas of the country, particularly in areas with moist sandy soil. Ecological reports have stated that some *Lindernia* species are saline tolerant and drought tolerant. Normally they have a long flowering period with the most profuse flowering from July to March (Shinha, 1987).

The flowers are very similar to wishbone flower or *Torenia* sp., which are now popular as an ornamental plant. *Lindernia* is not yet well known. Its flowers are small, and the genetic variability available in nature is not great enough to select promising varieties for use as a potted plant or groundcover. If a new variety could be produced with larger flowers and leaves and shorter internodes with a less erect habit, it would be a very useful plant for landscaping, providing a hardy and attractive ground cover or creeping potted plant.

Colchicine is a substance that has been used in tissue culture to induce chromosome doubling in many plant species. Polyploid plants often have the advantage of having larger organs such as thicker leaves and larger flowers than the haploid progenitors. Because pure colchicine is dangerous, for this research gout medication containing colchicine was used. This type of gout medication has been successfully used to induce mutations in other plant species before and it is much safer. However, the optimum concentration of colchicine to induce polyploidy or other mutations depends on the plant species and the type of plant tissue used. The purpose of this research was to determine the most appropriate concentration and duration of colchicine exposure to induce polyploidy in *Lindernia* explants *in vitro* for the benefit of creating greater genetic variability. This would provide a source of more diverse germplasm for future breeding programs and could result in a value added variety of *Lindernia* with a novel plant form or flower color for commercial exploitation.

2. Materials and methods

Nodes and leaf sections were excised from one-month-old *in vitro* Lindernia plantlets and placed in colchicine solution at the concentrations of 0, 5, 10, 15 and 20 ppm colchicine, agitated constantly on a shaker table at 120 rpm for durations of 1, 2 and 3 days. The experimental design was factorial in CRD with 15 treatments, 2 replicates each, and each replicate consisted of 10 explants. After the colchicine treatment, the explants were removed from the solution and excess colchicine solution was blotted off before the explants were inoculated onto semi-solid half-strength MS medium (Murashige and Skoog, 1962). After 30 days the survival rate was

recorded, as well as the number of new shoots generated from each explant. Putative polyploids were selected by screening for morphological variations such as darker green leaves, larger leaves, thicker leaves, more sharply serrate leaf margins, thicker stems, shorter internodes, and thicker roots. The putative polylploids were subcultured to semi-solid half-strength MS medium and grown for another 30 days, after which the plant height, leaf size, number of roots and root length were recorded. Next, for polyploidy frequency calculation, the plants with morphological variations visible in every branch were removed from the culture vessels and acclimatized in the greenhouse. The survival rate after acclimatization was recorded.

3. Results and Discussion

3.1 Effects of colchicine concentration and exposure time on survival rate

When node and leaf sections of Lindernia were exposed to colchicine from gout medication at the concentrations of 0, 5, 10, 15 and 20 ppm for 1, 2 and 3 days and then cultured on semi-solid half-strength MS medium for 30 days, the mean survival rate of the node and leaf explants tended to decrease with increasing concentrations of colchicine and increasing exposure time. Colchicine not only affects cell division, but is diffused to various cellular components in the plant tissues and can have a toxic effect at high concentrations (Dermen, 1940). Colchicine has an effect on the viscosity of the cytoplasm, which causes irregularities in cell function (Cook and London, 1952) including a doubling of chromosomes. In some cases this leads to an imbalance that results in cell death (Distabanjong *et al.*, 2007). In the present study, nodes and cells exposed to colchicine at the lowest concentration of 5 ppm and for the shortest exposure time of 1 day had a mean survival rate of 100%, which was the same as the control group. Comparing the survival rates of node sections versus leaf sections, over the different colchicine concentrations and exposure times, overall the node sections had a higher survival rate than leaf sections (Table 1). Considering the survival rates of node sections in different treatment groups, it was interesting to note that with an exposure time of 3 days, the survival rate of node sections exposed to colchicine at the concentrations of 15 ppm and 20 ppm was actually higher than the survival rate of those exposed to colchicine at the concentration of 10 ppm. This could be due to a build-up of tolerance to the colchicine. A similar case was reported in the work of Anyanee and Sombong (2010), who found that when *Anthurium andraeanum* cv. Micky Mouse was exposed to colchicine at the concentration of 0.2%, the survival rate was higher than for the treatment groups exposed to a colchicine concentration of 0.05% and 0.1% for the same duration. In an experiment on Alocasia, Thao *et al.* (2003) found that with exposure times of both 24 hours and 72 hours, Alocasia explants exposed to 0.05% colchicine had a higher survival rate than the explants exposed to 0.01% colchicine. However, it is possible that this variation in

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survival rate could be an anomaly observed when the number of replications was rather low, so it would be fruitful to repeat the experiment with a higher number of replications.

Table 1 : Survival rate of Lindernia node and leaf explants that were treated with different concentrations of colchicine for different durations and then cultured on semi-solid half-strength MS medium for 30 days *in vitro*

Colchicine concentration (ppm)	Exposure time (days)	Starting number of explants		Survival rate (%)	
		Node	Leaf	Node	Leaf
0	1	20	20	100	100
	2	20	20	100	100
	3	20	20	100	100
5	1	20	20	100	100
	2	20	20	95	75
	3	20	20	90	60
10	1	20	20	95	95
	2	20	20	95	60
	3	20	20	60	50
15	1	20	20	85	80
	2	20	20	90	70
	3	20	20	65	55
20	1	20	20	80	80
	2	20	20	75	65
	3	20	20	70	45

3.2 Growth of Lindernia plants regenerated from node and leaf sections treated with colchicine comparing to the control

3.2.1 Plant height and internode length

Shoots were regenerated from the surviving explants from all the treatment groups and cultured on semi-solid half-strength MS medium for another 30 days. Sixty days after colchicine treatment, the plant height and internode lengths were recorded and it was found that plantlets regenerated from colchicine-treated explants were smaller than those in the control group. Plantlets from the 5 ppm colchicine treatment group had the lowest mean plant height at 3.29±0.43 cm for those regenerated from node explants and 3.61±0.70 cm for those regenerated from leaf sections, respectively. Comparing exposure times, the plantlets regenerated from explants exposed to colchicine for 1 day had the lowest mean plant height at 3.39±0.52 cm (node) and 3.77±0.65 cm (leaf), respectively. Similarly, the mean internode lengths of plantets regenerated from node and leaf explants exposed to all colchicine treatments were shorter than those in the control group. Again, the shortest mean internode length was observed in the 5 ppm colchicine treatment group at 0.87±0.18 cm in plantlets derived from node sections and 0.86±0.25 cm in plantlets derived from leaf sections, respectively (Table 2). In addition, shorter exposure time was associated with shorter mean internode length. This indicates that colchicine had an effect on plant height, compactness and sturdiness, because the stems of plants regenerated from colchicine-treated explants tended to have thicker stems than those of the control group (Figure 1).

Table 2: Mean plant height and internode length at 60 days of *in vitro* Lindernia plants regenerated from node and leaf sections exposed to different concentrations of colchicine for different lengths of time.

Treatment	Plant height (cm)		Internode length (cm)	
	Node	Leaf	Node	Leaf
A: Colchicine concentration (ppm)				
0	3.69 ±0.36[b1/]	4.45 ±0.68[c]	1.15±0.14[c]	1.32±0.16[c]
5	3.29 ±0.43[a]	3.61 ±0.70[a]	0.87±0.18[a]	0.86±0.25[a]
10	3.64 ±0.83[b]	4.27 ±1.01[bc]	0.88±0.18[a]	0.97±0.20[b]
15	3.86 ±0.52[b]	3.99 ±0.54[b]	0.99±0.21[b]	0.96±0.18[b]
20	3.61 ±0.66[b]	3.95 ±0.99[b]	0.99±0.21[b]	0.96±0.22[b]
F-test	**	**	**	**
B: Exposure time (days)				
1	3.39 ±0.52[a]	3.77 ±0.65[a]	0.88±0.17[a]	0.93±0.27[a]
2	3.75 ±0.42[b]	4.14 ±0.84[b]	0.97±0.16[b]	1.07±0.20[b]
3	3.73 ±0.77[b]	4.25 ±0.96[b]	1.08±0.24[c]	1.04±0.27[b]
F-test	**	**	**	**
A × B	**	* *	**	**
C.V. (%)	16.85	20.99	21.43	25.74

[1/] Mean values in the same column followed by different superscripts are statistically different when compared using Duncan's New Multiple Range Test (DMRT)

** Statistically significant difference at 99% confidence level.

3.2.2 Leaf size

As shown in Table 3, significant differences were detected in the leaf width and leaf length of Lindernia plants regenerated from the different treatment groups. The shortest mean leaf width and leaf length was observed in the control group, in plants regenerated from both node sections and leaf sections. The plants regenerated from node and leaf explants exposed to colchicine at every concentration and for every duration had leaves that were almost twice as wide and long as the leaves of plants in the control group. This demonstrates that colchicine treatment can result in increased leaf size in Lindernia (Figure 1). Considering the combined influence of colchicine concentration and exposure time on leaf width and leaf length of Lindernia plants regenerated from treated node and leaf sections, highly statistically significant differences were detected (Table 3).

3.2.3 Number of roots and root length

Statistically significant differences were also noted in mean number of roots and mean root length of Lindernia plants regenerated from node and leaf explants subjected to different colchicine treatments. The control group had the highest mean number of roots and the highest mean root length. The colchicine exposure time also had an effect on the mean number of roots and root length. That is, the plants regenerated from node and leaf sections from the treatment groups with longer exposure time tended to have more roots and longer roots. When the combined effect of colchicine concentration and exposure time were analyzed, statistically

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significant differences in number of roots and root length were found in Lindernia plants regenerated from both node and leaf sections (Table 4). Our results indicate that colchicine. treatment resulted in plants with fewer and shorter roots than the control, but roots that appeared thicker and stronger than the control (Figure 2).

Table 3: Mean leaf width and length at 60 days of *in vitro* Lindernia plants regenerated from node and leaf sections exposed to different concentrations of colchicine for different lengths of time

Plant height (cm)	Internode length (cm)		Leaf length (cm)	
	Node	Leaf	Node	Leaf
A: Colchicine concentration (ppm)				
0	0.23 ±0.03[a1/]	0.22 ±0.02[a]	0.53±0.07[a]	0.53±0.08[a]
5	0.41 ±0.05[b]	0.46 ±0.12[b]	0.67±0.12[b]	0.62±0.13[b]
10	0.39 ±0.08[b]	0.43 ±0.10[b]	0.70±0.41[b]	0.70±0.13[c]
15	0.42 ±0.06[b]	0.43 ±0.09[b]	0.68±0.09[b]	0.69±0.10[c]
20	0.40 ±0.07[b]	0.44 ±0.10[b]	0.67±0.10[b]	0.71±0.12[c]
F-test	**	**	**	**
B: Exposure time (days)				
1	0.38 ±0.09[b]	0.41 ±0.12	0.65±0.12	0.63±0.13[a]
2	0.38 ±0.09[b]	0.40 ±0.12	0.62±0.10	0.64±0.11[ab]
3	0.35 ±0.11[a]	0.38 ±0.14	0.68±0.33	0.68±0.15[b]
F-test	*	ns	ns	*
A × B	**	**	**	**
C.V. (%)	24.32	32.50	32.31	20.00

[1/]Mean values in the same column followed by different superscripts are statistically different when compared using Duncan's New Multiple Range Test (DMRT)
ns no statistically significant difference
* Statistically significant difference at 95% confidence level
** Statistically significant difference at 99% confidence level

Table 4: Mean number of roots and root length at 60 days of *in vitro* Lindernia plants regenerated from node and leaf sections exposed to different concentrations of colchicine for different lengths of time.

Treatment	Number of roots		Root length (cm)	
	Node	Leaf	Node	Leaf
A: Colchicine concentration (ppm)				
0	23.25±4.91[c1/]	26.35±4.15[b]	2.53±0.35[c]	2.41±0.31[b]
5	12.20±2.59[a]	13.04±3.65[b]	1.73±0.28[a]	2.10±0.45[a]
10	12.46±3.69[ab]	12.97±2.90[a]	1.86±0.47[ab]	2.19±0.28[a]
15	13.85±2.30[b]	12.42±2.48[a]	1.97±0.29[b]	2.15±0.30[a]
20	12.61±2.67[ab]	11.66±3.34[a]	1.86±0.38[ab]	2.19±0.41[a]
F-test	**	**	**	**
B: Exposure time (days)				
1	14.05±4.16[a]	14.61±5.59[a]	1.92±0.37[a]	2.13±0.33[a]
2	14.36±4.02[a]	14.66±5.37[a]	1.92±0.40[a]	2.23±0.29[ab]
3	16.22±7.21[b]	16.59±8.05[b]	2.11±0.55[b]	2.27±0.45[b]
F-test	**	**	**	*
A × B	**	**	**	**
C.V. (%)	36.25	42.38	22.61	16.74

[1/]Mean values in the same column followed by different superscripts are statistically different when compared using Duncan's New Multiple Range Test (DMRT)
* Statistically significant difference at 95% confidence level
** Statistically significant difference at 99% confidence level

The growth characteristics of Lindernia plants following colchicine treatment may be explained by a delay or slowed down pace of cell division due to the effects of colchicine, followed by a resumption of the normal rate of cell division later (Siranuch, 1997). Another explanation is that when colchicine induces chromosome doubling, the plant organs developing from tissue with tetraploid cells might have altered morphology due to larger cell size, even if the total number of cells is less and the overall growth rate is slower (Nopporn, 2003; Chandrasekharan *et al.*, 1975). Our findings were consistent with those of Santida (2009), who found that when hybrid Torenia (a cross between a commercial variety of *Torenia concolor* and *T. fournieri*) and native wild Torenia were exposed to colchicine from gout medication, the plant height, spread and number of branches tended to drop with increased concentration of colchicine and increased exposure time, while the stem width, leaf size and leaf thickness tended to increase. In a study on white-flowered *Spathoglottis plicata*, Weerapatra (2009) found that every concentration of colchicine tested caused the plant height, leaf length, number of roots and root length to decrease while only the leaf width increased.

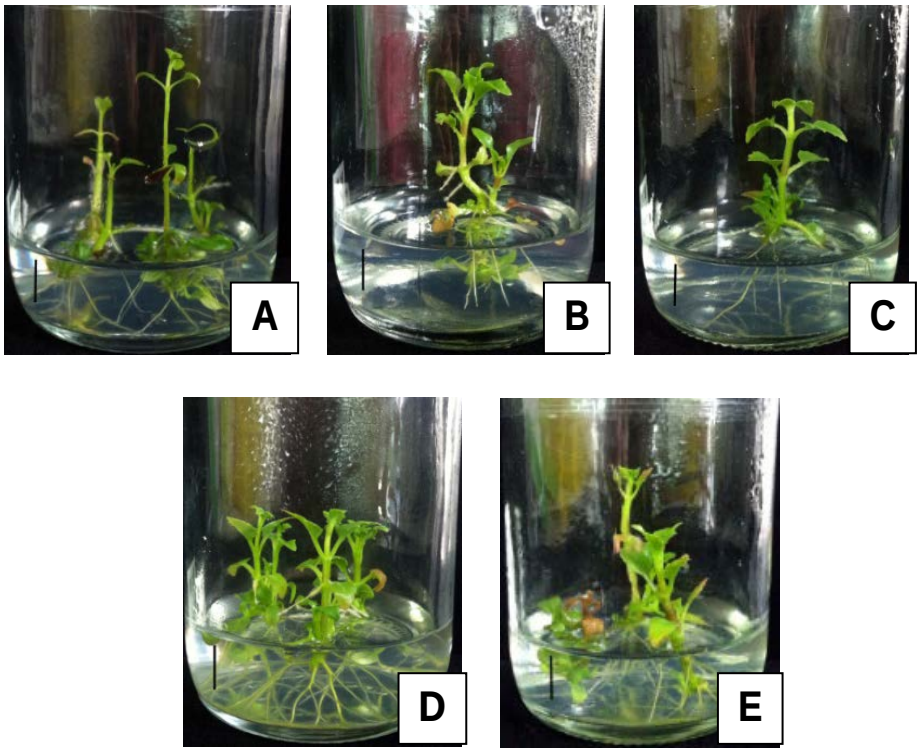

Figure 1 Lindernia plants regenerated from node and leaf sections exposed to 5 different concentrations of colchicine
(A) no colchicine (control) (B) 5 ppm colchicine
(C) 10 ppm colchicine (D) 15 ppm colchicine
(E) 20 ppm colchicine

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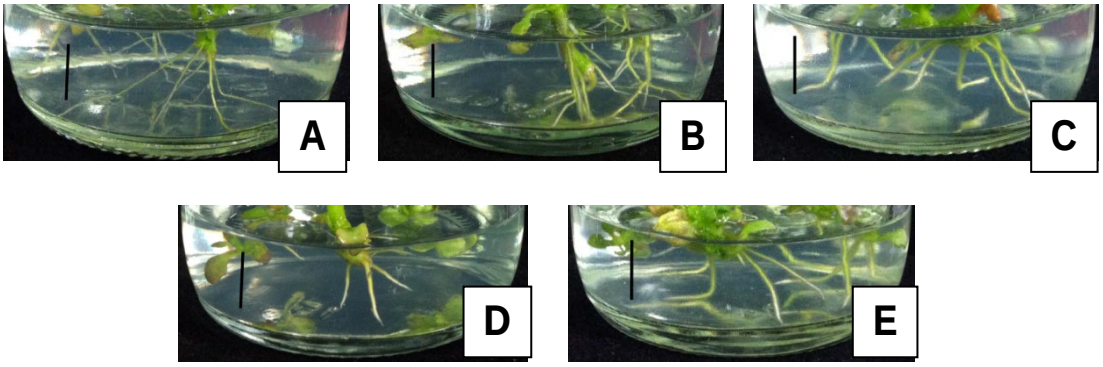

Figure 2 Roots of Lindernia plants regenerated from node and leaf sections exposed to 5 different concentrations of colchicine
(A) no colchicine (control) (B) 5 ppm colchicine
(C) 10 ppm colchicine (D) 15 ppm colchicine
(E) 20 ppm colchicine

3.3 Frequency of polyploidy induction

Our results showed that Increasing concentration of colchicine and increasing exposure time led to a reduction in the ability of Lindernia node and leaf explants to regenerate new shoots. This is probably because colchicine does not only affect cell division, but spreads to different parts of the cell causing a toxic effect that may inhibit the growth and organ regeneration ability of the plant (Dermen, 1940). However, exposure to colchicine at every concentration tested and for every duration tested did result in polyploidy induction (Table 4). The treatment group exposed to colchicine at the concentration of 10 ppm for 3 days had the lowest survival rate at 50–60% (Table 1), but also the highest frequency of polyploidy induction at the rate of 22.00% for node explants and 16.22% for leaf explants, respectively. It is possible that each individual cell has a different response to colchicine, so that only certain cells or groups of cells in a plant change to tetraploids in response to colchicine (Wimol, 1984). Dermen (1940) wrote that in cases where colchicine treatment does not result in polyploidy at all, it is likely because the colchicine was not able to diffuse through the cells to the target tissues, or else because the tissue in question has a high level of resistance to colchicine. Comparing the response of node explants and leaf explants in the present study, we found that polyploidy plants were more likely to be regenerated from node sections than from leaf (Table 5). Normally, polyploidy induction using colchicine should be performed on quickly growing tissues with a large number of dividing cells. The most suitable plant parts are seeds, sprouts and growing meristems. Node section explants probably displayed a higher frequency of polyploidy because the axial buds contain rapidly growing tissues.

3.4 Survival rate after planting out of putative polyploidy plantlets

The putative polyploidy plants were selected based on morphological changes that made them different from the control group, such as darker green leaves, larger and thicker leaves,

Table 5: Frequency of polyploidy found in Lindernia plantlets regenerated from node and leaf sections that were exposed to different concentrations of colchicine for different lengths of time.

Colchicine concentration(ppm)	Exposure time (days)	Number of new shoots		Number of putative polyploids		Frequency of polyploidy (%)	
		Node	Leaf	Node	Leaf	Node	Leaf
0	1	386	306	0.00	0.00	0.00	0.00
	2	369	376	0.00	0.00	0.00	0.00
	3	306	396	0.00	0.00	0.00	0.00
5	1	282	252	34	14	12.06	5.56
	2	146	118	25	12	17.12	10.17
	3	182	92	11	6	6.04	6.52
10	1	261	212	43	11	16.47	5.19
	2	192	133	12	9	6.25	6.77
	3	50	37	11	6	22.00	16.22
15	1	217	152	26	17	11.98	11.18
	2	149	125	18	14	12.08	11.20
	3	99	76	20	11	20.20	14.47
20	1	235	221	33	15	14.04	6.79
	2	144	132	22	15	15.28	11.36
	3	101	38	11	6	10.89	15.79

Table 6: Survival rate after planting out of Lindernia plants that were regenerated from node and leaf sections.

Colchicine concentration (ppm)	Exposure time (days)	Survival rate after planting out (%)	
		Node explants	Leaf explants
0	1	100.00	100.00
	2	100.00	100.00
	3	100.00	100.00
5	1	96.00	78.57
	2	96.00	50.00
	3	100.00	33.33
10	1	88.00	72.72
	2	83.33	77.78
	3	90.90	50.00
15	1	96.00	82.35
	2	83.33	64.28
	3	90.00	45.45
20	1	100.00	53.33
	2	100.00	33.33
	3	90.90	0.00

more clearly indented leaf margins, thicker stems, shorter internodes and larger roots. The putative polyploids and control group plants were removed from the tissue culture vessels and planted in seed trays filled with peat moss. The control group, including both those plants regenerated from node sections and those regenerated from leaf sections, had the highest survival rate at 100%. For the colchicine-treated groups, the plants regenerated from node sections had a

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survival rate of 84–100% and the plants regenerated from leaf sections had a survival rate of 0 – 82% (Table 6). The plants regenerated from node sections tended to be stronger because only a single shoot would arise from the axial bud, whereas leaf tissue usually initiated several small shoots that had to compete for nutrients and growing space, resulting in smaller, weaker plants that had a lower survival rate after planting out.

4. Conclusion

Our work immersing *in vitro* node and leaf sections of *Lindernia* sp. in varying concentrations of colchicine solution prepared from gout medication tablets demonstrated that the initial survival rate of the node and leaf sections 30 days after colchicine exposure decreased with increasing concentration of colchicine and increasing exposure time. The treatment group that was exposed to 10 ppm colchicine solution for 3 days had the lowest survival rate at 60% for node sections and 50% for leaf sections, respectively. However, this same treatment group also had the highest frequency of polyploidy induction at 22.00% for node sections and 16.22% for leaf sections, respectively. Lindernia plants generated from the node and leaf sections treated with colchicine in every treatment group had shorter plant height, shorter internodes, shorter roots and fewer roots than the control group, but they had thicker stems, thicker roots and both wider and longer leaves than the control. The frequency of polyploidy induction was higher in node sections than in leaf sections, and the plants regenerated from node sections were also stronger and had a higher survival rate when planted out than those regenerated from leaf sections.

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