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Evaluation of *ex vitro* growth and field performance of micropropagated polyploid clones of *Neolamarckia cadamba*

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ABSTRACT

The evaluation of field performance in tissue culture-derived seedlings is essential to determine their suitability for reforestation programs and commercial forestry, as it provides valuable insights into their adaptability to different environmental conditions and management practices. This report provides the first analysis of the growth characteristics of colchicine-induced polyploids of *Neolamarckia cadamba*. Field planting revealed that octoploid plants grow slower than mixoploid and tetraploid plants, but SPAD results showed that they have better photosynthetic capacity than the other two types. Notably, after 30 months of transplantation, the polyploid clones of *N. cadamba* exhibited better growth performance than other *N. cadamba* trees planted in various locations. These novel polyploid clones of *N. cadamba* could be valuable resources for advanced breeding programs aimed at producing improved clones for planted forest development. By enhancing the adaptability of polyploid *N. cadamba* clones, they have the potential to reduce the site-specific effects of *N. cadamba*, ultimately improving tree productivity and adaptation across different planting sites.

1. INTRODUCTION

The field performance of *in vitro* regenerated seedlings is critical to consider when evaluating the success of tissue culture-derived planting materials. Tissue culture techniques have gained popularity due to their advantages over traditional seedling propagation methods. These techniques enable the production of large numbers of true-to-type seedlings with desirable traits [1,2]. Hence, it is essential to evaluate the field performance of tissue culture-derived seedlings to determine their suitability for reforestation programs and commercial forestry. Furthermore, it provides valuable insights into the adaptability of these *in vitro* regenerated seedlings to different environmental conditions and silvicultural practices, ultimately contributing to the development of sustainable and productive forestry practices [3].

Neolamarckia cadamba is traditionally propagated using seeds from the selected candidate plus trees, and planted forests are established by planting the seedlings [3,4]. Propagation using seeds is undesirable and leads to off-type plants appearing in the progeny due to cross-pollination and segregation. Furthermore, if the mother trees are infected with diseases, they are transmissible to the next generation, despite the application of fungicide or insecticide during the seed germination. *In vitro* propagation or micropropagation is the method of choice to palliate this problem by producing disease-free and quality planting materials from the elite mother trees in large numbers. In fact, quality planting materials can be generated in large volumes for any planting season with minimum use of space and less initial plant material compared to traditional propagation methods.

N. cadamba, a species commonly used in reforestation programs, has well-established protocols for micropropagation [1,5-7]. However, limited information is available regarding the field performance of micropropagated *N. cadamba* in Malaysia. This study aimed to determine the *ex vitro* growth and field performance of *N. cadamba*, focusing on tissue culture-derived seedlings. The results of this study will provide useful information for the development of reforestation programs using tissue culture-derived seedlings of *N. cadamba*.

2. MATERIALS AND METHODS

2.1. Plant Materials

The plant materials used in this study were derived from prior research conducted by Eng *et al*., [7]. Briefly, nodal segments of *N. cadamba*

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were subjected to various concentrations and durations of colchicine treatment, resulting in the development of plants exhibiting diverse ploidy levels, including mixoploids (2n + 4n = 4x + 8x) and octoploids (4n = 8x = 88). *N. cadamba* is a naturally occurring tetraploid plant with a chromosome count of 44 (2n = 4x = 44). The levels of polyploidy in *N. cadamba* were validated using flow cytometric and cytogenetic analyses [7].

2.2. *Ex vitro* Growth Performance

Acclimatized plantlets were transplanted to polybags 7' × 11' filled with soil mix consisting of soil, compost and sand in the ratio of 3:2:1. The medium was prepared before sterilization by autoclaving at 121°C for 20 min. The plantlets were watered daily and fertilized with 5 g inorganic fertilizer, N: P:K (15:12:11) monthly to stimulate growth. Growth data collected include plant height, stem girth, leaf length, and leaf width (second node), which were recorded once every 2 months for 6 months. This study covered tetraploid, mixoploid, and octoploid plants, with each genotype comprising ten replicates.

2.3. Field Transplantation

The field trial was conducted on a site near a plywood processing plant at Linshanhao Plywood (S) Sdn Bhd, Kuching, Sarawak. The acclimatized plantlets were transplanted into 7' × 11' polybags filled with compost produced from the boiler in the plywood processing plant and maintained at the nursery for 3 months. Afterward, the seedlings were transplanted to the cleared field for a field trial study. The planting hole (30 × 30 × 30 cm) at 5 × 4 m spacing was prepared before tree planting. Plants were rainfed and fertilized with commercial-grade fertilizer at the time of planting and 3–4 times/year after planting at 3 or 4-month intervals. The field trial was set up in a randomized complete block design. Each block consisted of all the treatments and controls for the experiment. The growth performance of seedlings, such as plant height, root collar diameter, stem diameter, leaf length and width (second node), and soil plant analysis development (SPAD) value, was monitored every 3 or 6 months. Data collected were subjected to analysis of variance (SPSS version 23 program), followed by the Duncan new multiple range test ($P = 0.05$).

3. RESULTS AND DISCUSSION

3.1. *Ex vitro* Growth Performance

Two months after the acclimatized plants were transferred to the soil mix, the mean stem girth of the mixoploid (0.40 ± 0.04 cm) and octoploid (0.41 ± 0.03 cm) plants was found to be higher than that of the tetraploid plants (0.34 ± 0.04 cm). However, no significant differences were observed for other recorded parameters, including mean plant height, mean leaf length, and mean leaf width, as indicated in Table 1 and Figure 1a. In the 4th month [Table 1 and Figure 1b], the mean height of the tetraploid plants (7.97 ± 0.10 cm) was significantly greater than that of the octoploid plants (7.46 ± 0.18 cm). In addition, the mean stem girth of the octoploid plants (0.41 ± 0.03 cm) was significantly larger than that of the tetraploid plants (0.34 ± 0.04 cm). However, no differences were observed for the other recorded parameters, namely, leaf length and width.

By the 6th month [Table 1 and Figure 1c], the mean height of the tetraploid plants (7.97 ± 0.10 cm) remained significantly higher than that of both the mixoploid (0.40 ± 0.04 cm) and octoploid (0.41 ± 0.03 cm) plants. No significant differences among the three genotypes were found in the mean stem girths. Furthermore, the mean leaf length of the mixoploid plants was higher than that of the tetraploid and octoploid plants, while the mean leaf width of the mixoploid plants was greater than that of the octoploid plants but showed no difference when compared to the tetraploid plants.

The *ex vitro* growth study observed that the octoploid plants had shorter stems than the tetraploid plants after the 4th and 6th months, although no significant difference was observed in the 2nd month

Table 1: *Ex vitro* growth of tetraploid, mixoploid, and octoploid *Neolamarckia cadamba* plants.

Parameters	Tetraploids	Mixoploids	Octoploids
After 2-month on soil mix			
Height (cm)±SE	7.97±0.10^{a}	7.64±0.26^{a}	7.46±0.18^{a}
Stem girth (cm)±SE	0.34±0.04^{b}	0.40±0.04^{a}	0.41±0.03^{a}
Leaf length (cm)±SE	7.46±0.17^{a}	7.17±0.33^{a}	6.99±0.24^{a}
Leaf width (cm)±SE	3.91±0.18^{a}	3.73±0.18^{a}	3.58±0.11^{a}
After 4-month on soil mix			
Height (cm)±SE	16.14±0.21^{a}	15.44±0.61^{ab}	14.36±0.64^{b}
Stem girth (cm)±SE	0.63±0.10^{b}	0.68±0.03^{ab}	0.69±0.02^{a}
Leaf length (cm)±SE	14.26±0.16^{a}	14.60±0.32^{a}	14.14±0.30^{a}
Leaf width (cm)±SE	8.61±0.21^{a}	8.98±0.22^{a}	8.58±0.18^{a}
After 6-month on soil mix			
Height (cm)±SE	33.85±0.62^{a}	28.72±0.85^{b}	22.64±0.83^{c}
Stem girth (cm)±SE	0.92±0.04^{a}	0.92±0.03^{a}	0.86±0.02^{a}
Leaf length (cm)±SE	20.18±0.55^{b}	21.67±0.45^{a}	19.38±0.32^{b}
Leaf width (cm)±SE	14.56±0.66^{ab}	15.28±0.40^{a}	14.05±0.55^{b}

The data are means of 10 replicates per treatment. Within each row, means with different letters indicate significantly different by Duncan new multiple range test at probability 0.05.

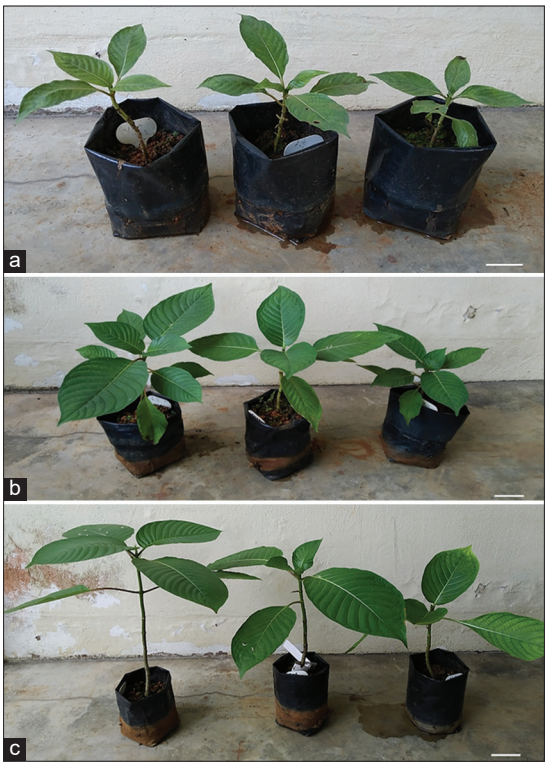

Figure 1: *Ex vitro* growth of tetraploid (left), mixoploid (middle), and octoploid (right) of *Neolamarckia cadamba* plants after 2 months (a), 4 months (b), and 6 months (c) on soil mix. Bar = 4 cm.

[Table 1 and Figure 1]. The mean stem girth of the octoploid plants was greater than that of the tetraploid plants in the 2nd and 4th months, but no difference was found in the 6th month [Table 1]. This slower growth development pattern was consistently observed in induced polyploids of *N. cadamba* compared to their progenitors.

Based on morphological data compiled in a review article, out of 18 different species of artificial polyploids, 12 exhibited reduced plant height, four showed increased height, and two remained unchanged compared to their progenitors [8]. These findings suggest that colchicine-induced polyploidization predominantly reduces plant height, while only a small number of species can maintain or increase their height.

Many colchicine-induced polyploids of woody trees show reduced growth rate by producing shorter trees in Gymnosperms [9], *Paulownia tomentosa* [10], *Acacia crassicarpa* [11], *Eriobotrya japonica* [12], *Acacia mangium* [13], and *Salix viminalis* [14]. According to Griffin *et al*., [13], the slow growth of induced polyploid *A. mangium* poses challenges for growers, particularly when high production volume is a priority. To address this issue, further breeding efforts should focus on producing the next generations, such as triploid and tetraploid hybrids, to overcome the growth limitations.

While *N. cadamba* experienced reduced growth under both *in vitro* [7] and *ex vitro* conditions, it did not exhibit signs of poor plant health, stunted growth, or mortality. Despite not meeting expected quality improvements, several colchicine-induced polyploids remain valuable as germplasm resources for future breeding programs [15].

Several factors cause delayed growth when the ploidy level of the plant increases. First, in a study involving *Arabidopsis thaliana*, different ploidy levels (diploid - 2n, tetraploid - 4n, hexaploid - 6n, and octoploid - 8n) were phenotypically characterized to assess their growth rates. Polyploid plants exhibited slower development compared to diploid plants, with octoploid plants specifically showing delayed flowering. Further, analysis of their leaves revealed that as the ploidy level increased, cell size increased while cell density decreased. With fewer cells available, the growth will be reduced [16].

Second, delayed growth in polyploids can be limited by nitrogen and phosphorus availability in the growing environment, as these elements are essential components of chromosomes [17,18]. Third, cells with a bigger genome require more time to complete a cell division cycle, particularly during the synthesis phase (S), where they spend a longer time reaching a larger size before they can divide [19]. Finally, the regulation of cell proliferation plays a significant role in determining the size of an organ, as the total number of cells and their sizes collectively determine the size of an organ [20].

Table 2: Plant growth of tetraploid, mixoploid and octoploid *Neolamarckia cadamba* plants after about 3 months in the field.

Parameters	Tetraploids	Mixoploids	Octoploids
Height (cm)±SE	50.97±2.97^{a}	44.19±1.96^{b}	33.71±2.30^{c}
Root collar diameter (cm)±SE	1.40±0.06^{a}	1.29±0.04^{a}	0.98±0.06^{b}
Leaf length (cm)±SE	33.43±1.43^{a}	30.35±1.03^{a}	25.84±1.57^{b}
Leaf width (cm)±SE	23.53±1.13^{a}	22.85±0.85^{a}	18.93±1.21^{b}
SPAD value±SE	32.82±0.68^{b}	33.67±0.53^{b}	39.02±0.98^{a}

Within each row, means with different letters indicate significantly different by DMRT at probability 0.05.

3.2. Field Transplantation

In total, 15 tetraploids, 36 mixoploids, and 29 octoploids were transplanted into the field after being kept in the nursery for 3 months. After about 3 months in the field, the tetraploid plants were found to be significantly taller (50.97 cm), followed by the mixoploid plants (44.91 cm), and the octoploid plants were the shortest at 33.71 cm [Table 2]. In terms of measurements for root collar diameter, leaf length, and leaf width, the octoploid plants had smaller mean values than the mixoploid and tetraploid plants, as shown in Table 2. Contrastingly, the mean SPAD value was significantly higher in octoploid plants (39.02) compared to both mixoploid (33.67) and tetraploid (32.82) plants. A portable chlorophyll meter (TYS-B, Mindful Technology Co. Ltd), equipped with two light sources (red light at 650 nm and infrared light at 940 nm), was used to measure the SPAD values of the leaves. SPAD values correlate positively with the chlorophyll content, and an increased chlorophyll content in plants may indicate an increased rate of photosynthesis. Based on the results [Table 2], it is predicted that the polyploid plants will have a higher photosynthetic capacity due to their higher SPAD value or higher content of chlorophyll. This finding agrees with a previous study using *in vitro* cultures of *N. cadamba* [7]. Figure 2 shows the field assessment of *N. cadamba* polyploid clones after 6 months of transplanting in the field.

In the current study, the growth characteristics of polyploid clones (mixoploids and octaploids) were compared to control plants and colchine-treated tetraploid plants. *N. cadamba* is naturally a tetraploid. The 3-month and 30-month field trial results showed that *N. cadamba* octoploid plants exhibited slower growth than mixoploid and tetraploid plants [Figure 3], as indicated by various recorded growth parameters,

Table 3: Growth performance of tetraploid, mixoploid, and octoploid *Neolamarckia cadamba* plants after 30 months in the field.

Parameters	Control	Colchicine-treated plants		
		Tetraploids	Mixoploids	Octoploids
Mean dbh (cm)±SE	18.82±1.77^{ab}	20.47±1.50^{ab}	16.94±0.54^{b}	13.93±0.57^{c}
dbh MAI (cm/year)	7.53	8.19	6.78	5.57
Max. dbh (cm)	21.90	28.90	30.00	19.40
Mean height (m)±SE	12.58±1.26^{ab}	14.20±1.02^{ab}	10.11±0.36^{b}	7.33±0.19^{c}
Height MAI (m/year)	5.03	5.68	4.04	2.93
Max. height (m)	15.00	22.72	17.70	9.50
Mean basal area (m^2)±SE	0.029±0.004^{ab}	0.035±0.005^{ab}	0.023±0.002^{b}	0.016±0.001^{c}
Mean volume (m^3)±SE	0.184±0.034^{ab}	0.260±0.047^{ab}	0.119±0.015^{b}	0.058±0.006^{c}

Within each row, means with different letters indicate significantly different by DMRT at probability 0.05.

Table 4: Comparison of dbh and height of *Neolamarckia cadamba* (Kelampayan/Jabon/Laran) trees of different ages at various Kelampayan planting sites in Malaysia, Indonesia, and China.

Location	Species/type	Age (month)	Mean dbh (cm)	Mean height (cm)	References
Kelampayan Trial Plot, Sejingkat (5×4 m)	Control	30	18.82	1,258.00	This study
	Tetraploids	30	20.47	1,419.60	
	Mixoploids	30	16.94	1,011.11	
	Octaploids	30	13.93	732.76	
LPF/0032 (5×5 m)	Kelampayan	24	10.30	668.00	Ho *et al*., [3]
LPF/0032 (3×3 m)	Kelampayan	24	10.54	723.00	
LPF/0032 (5×5 m)	Kelampayan	36	12.00	800.00	
LPF/0032 (3×3 m)	Kelampayan	36	12.60	839.00	
Sukadamai-3, West Java, Indonesia. (2.5×2.5 m)	White Jabon	18	5.62	450.00	Seo *et al*., [22]
Sukadamai-2, West Java, Indonesia (2.5×2.5 m),	White Jabon	24	7.62	619.00	
Segaliud Lokan, Sabah (3×3 m)	Laran	18	8.96	740.00	Ajik [23]
	Laran	24	10.87	937.00	
	Laran	30	11.60	1,059.00	
	Laran	36	12.90	1,115.00	
South Subtropical Region of China (3×3 m)	Clone BN1, Plot 1	18	11.30	780.00	Wei and Zhu, [24]
	Clone BN1, Plot 12	30	13.00	940.00	
	Clone BN1, Plot 4	30	14.10	1,180.00	
	Clone BN1, Plot 1	30	16.80	1,260.00	

Figure 2: Field assessment of *Neolamarckia cadamba* polyploid clones (6-month-old). (a) Mixoploid (dbh = 3.9 cm); (b) Tetraploid (dbh = 4.3 cm); and (c) Octoploid (dbh = 3.4 cm).

Figure 3: Field assessment of *Neolamarckia cadamba* polyploid clones (30-month-old). (a) Control (dbh = 21.9 cm); (b) Tetraploid (dbh = 28.9 cm); (c) Mixoploid (dbh = 30.0 cm); and (d) Octoploid (dbh = 19.4 cm).

such as plant height, root collar diameter, stem diameter, leaf length, and leaf width [Table 3]. These trends were similarly observed in both *in vitro* and *ex vitro* growth experiments of *N. cadamba*, where octoploid plants showed slower growth than the tetraploid and mixoploid plants. However, the SPAD value for *N. cadamba* octoploid plants was found to be higher than that of the mixoploid and tetraploid plants, as shown in Table 2. In comparison, the colchicine-treated *N. cadamba* plants have a relatively higher growth performance after 30 months of transplantation

than other *N. cadamba* trees over different planting sites. However, we cannot predict the long-term growth and adaptability of these polyploid clones beyond the reported timeframe. We are monitoring the growth and adaptability of these clones at 6-month intervals. Table 4 compares the dbh and height of *N. cadamba* trees at various planting sites in Malaysia, Indonesia, and China.

Polyploidization is often praised for its capability to manifest "gigantism" traits in various organs, thus providing greater productivity and economic return [15]. However, not all polyploids exhibit gigantism in the same manner, as some exhibit bigger plants while others do the opposite. The effects of increased ploidy levels on morphological, physiological, cellular, and biochemical aspects cannot always be predicted [21].

As previously discussed by Eng *et al*., [7], induced polyploids may grow slower than their progenitors due to three factors: reduction of cell density as a result of greater cell size, limited nitrogen and phosphorus elements in the soil that are building blocks of chromosomes, and longer time required to complete a complete cycle of mitosis in induced polyploid cells. Although the number of chromosomes has doubled in polyploids, they are not necessarily twice as productive, vigorous, and resistant as their progenitors [15]. Furthermore, the expected outcomes may not even prevail with polyploidization success [15,21].

4. CONCLUSION

This is the first report on the growth characteristics of colchicine-treated polyploids of *N. cadamba*. In terms of *ex vitro* or field planting, octoploid plants grow slower than mixoploid and tetraploid plants. However, SPAD results show that octoploid plants have a better photosynthetic capacity than mixoploid and tetraploid plants. Nevertheless, after 30 months of transplantation, the polyploid clones of *N. cadamba* showed relatively better growth performance than other *N. cadamba* trees planted in various sites across Malaysia, Indonesia, and China. These novel polyploid clones of *N. cadamba* could be valuable resources for genetic improvement programs, such as the production of hexaploid (2n = 6x = 66) plants by intercrossing between *N. cadamba* octoploid (2n = 8x = 88) and tetraploid (2n = 4x = 44). The creation of polyploid clones of *N. cadamba* has the potential to mitigate the adverse effects of site-specific challenges, as it has been reported elsewhere that *N. cadamba* is a site-specific species and grows best along river banks and riparian areas and sub-optimally at higher terrain. Therefore, these clones may have the potential to adapt and grow best in higher terrain or altitude, ultimately improving tree productivity and adaptation across various planting sites.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All data obtained or analyzed in this study are included in this manuscript.

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