In vitro induction and identification of polyploid *Neolamarckia cadamba* plants by colchicine treatment

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ABSTRACT

Polyploidization has played a crucial role in plant breeding and crop improvement. However, studies on the polyploidization of tropical tree species are still very scarce in this region. This paper described the *in vitro* induction and identification of polyploid plants of *Neolamarckia cadamba* by colchicine treatment. *N. cadamba* belongs to the Rubiaceae family is a natural tetraploid plant with 44 chromosomes ($2n = 4x = 44$). Nodal segments were treated with colchicine (0.1%, 0.3% and 0.5%) for 24 h and 48 h before transferring to shoot regeneration medium. Flow cytometry (FCM) and chromosome count were employed to determine the ploidy level and chromosome number of the regenerants, respectively. Of 180 colchicine-treated nodal segments, 39, 14 and 22 were tetraploids, mixoploids and octoploids, respectively. The highest percentage of polyploidization (20% octoploids; 6.7% mixoploids) was observed after treated with 0.3% colchicine for 48 h. The DNA content of tetraploid (4C) and octoploid (8C) was 2.59 ± 0.09 pg and 5.35 ± 0.24 pg, respectively. Mixoploid plants are made up of mixed tetraploid and octoploid cells. Chromosome count confirmed that tetraploid cell has 44 chromosomes and colchicine-induced octoploid cell has 88 chromosomes. Both octoploids and mixoploids grew slower than tetraploids under *in vitro* conditions. Morphological characterizations showed that mixoploid and octoploid leaves had thicker leaf blades, thicker midrib, bigger stomata size, lower stomata density, higher SPAD value and smaller pith layer than tetraploids. This indicates that polyploidization has changed and resulted in traits that are predicted to increase photosynthetic capacity of *N. cadamba*. These novel polyploid plants could be valuable resources for advanced *N. cadamba* breeding programs to produce improved clones for planted forest development.

Subjects Agricultural Science, Biotechnology, Plant Science

Keywords *Neolamarckia cadamba*, Planted forest, *In vitro* culture, Polyploidization, Colchicine, Polyploid, *Anthocephalus cadamba*, Flow cytometry, Octoploid, Mixoploid

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INTRODUCTION

Neolamarckia cadamba (Roxb.) Bosser or *Anthocephalus cadamba* Roxb synonym is a fast-growing multipurpose tree, self-pruning and growing vigorously in exploited areas, such as logged-over forests and plantations (*Krisnawati, Kallio & Kanninen, 2011*; *Ho, Pang & Julaihi, 2014*; *Nordahlia et al., 2014*; *Ho, Ling & Pang, 2020*). It belongs to the Rubiaceae

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family and is a natural tetraploid plant with 44 chromosomes (2n = 4x = 44) (*Bedi, Bir & Gill, 1981*; *Kienh & Lorence, 1996*; *Puangsomlee & Puff, 2001*). It is a lightweight hardwood with a density of 290–560 kg/m^3 at 15% moisture content. The wood has a moderately fine to medium texture that is easy to work with hand and machine tools to give a perfect surface. This makes it an excellent raw material for plywood production, light construction work and furniture manufacturing (*Jøker, 2000*; *Lim & Chung, 2002*; *Orwa et al., 2009*; *Lal et al., 2010*). It is also suitable for reforestation and enrichment planting schemes (*Krishnapillay, Razak & Appanah, 2007*). *N. cadamba* tree bark has also been used in traditional medicine to treat various illnesses (*Mondal et al., 2009*). Despite being a commercially important commodity, attempts to improve *N. cadamba* have been limited to gene mutation study using ethyl methanesulphonate (EMS) (*Zayed et al., 2014*) and physical mutagens, such as gamma rays (*Zanzibar & Danu, 2015*). Mutation at ploidy level or polyploidization has yet to be studied.

Colchicine is an inhibitor to polymerization of the tubulin in nucleus that can cause polyploidization in cells. It binds with β-tubulin in the microtubule, resulting in disassociation and destabilization of the microtubule (*Lu et al., 2012*). Microtubule enables translocation of chromosomes during cell division (*Hammond, Cai & Verhey, 2008*). The formation of polyploid is due to the failure of chromosome pairs to separate during the anaphase of mitosis as mitosis ceases at metaphase. This will result in two sets of chromosomes in a single cell or the formation of polyploid cells (*Inoue, 1952*; *Eigsti & Dustin, 1956*). To date, colchicine is widely used in polyploid induction with different explants, concentrations, treatment durations and methods (*Hassan et al., 2020*; *Julião et al., 2020*; *Li et al., 2020*; *Zhang & Gao, 2020*). Polyploidization of tropical woody tree species has been rare due to slow-growing habits, long life cycles and lack of *in vitro* regeneration protocols.

The evolution and formation of new plant species are caused by natural and gradual adaptation to changing environments over a long period of time. Polyploidization increases the number of dominant alleles, diminishing the effects of recessive alleles that often result in undesirable traits (*Comai, 2005*; *Soltis et al., 2015*). This hypothesis makes polyploidization an attractive option for plant breeding to produce superior plants. Some induced polyploids were proven to adapt and tolerate better than their progenitors under undesirable or hostile environments. The induced tetraploid of *Plumbago auriculata* can tolerate cold environment at −5 °C for 24 h (*Jiang et al., 2020*). In another study, the seed production of *Themeda triandra* tetraploid was quadrupled and heavier than its diploid under a very dry and hot arid environment (*Godfree et al., 2017*). The acquired "giga" effects in polyploids increase the size of the cells, followed by doubling in chromosome numbers (*Sattler, Carvalho & Clarindo, 2016*). This "giga" effect may contribute to greater agronomical gains in polyploid plants by producing larger organs like flowers, fruits, leaves, stems, roots, etc. The fruits of *Actinidia chinensis* tetraploid increase by 50–60% in size compared with those of the diploid (*Wu et al., 2012*), while in *Thymus vulgaris* tetraploid, an increase in the essential oil extracted from the plants was observed (*Shmeit et al., 2020*). Polyploidization is vital in the creation of triploid plants either within species or between species. Triploid plants are high in demand, especially in the fruit and vegetable industries where seedless