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Research Article

Cytogenetic, chromosome count optimization and automation of *Neolamarckia cadamba* (Rubiaceae) root tips derived from *in vitro* mutagenesis

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Abstract

Chromosome count is the only direct way to determine the number of chromosomes of a species. This study is often considered trivial that seldom described and discussed in detail. Therefore, it is inevitable that the chromosome count protocol should be revised and revisited before it becomes obliterated. In the present study, we encountered challenges in obtaining a clear micrograph for the chromosome count of active mitotic cells of *Neolamarckia cadamba* (Roxb.) Bosser (Rubiaceae) root tips. Several obstacles were determined through micrograph observation, such as existing unwanted particles in cells, poor chromosome staining and chromosome clumping. To overcome these, root tip types, staining methodologies, squashing methods were among the factors assessed to obtain clear micrographs. The chromosome counts of *N. cadamba* under optimized procedure showed $2n = 44$ chromosomes. We also apply digital technology in chromosome counts, such as online databases and graphic software that are open source and freely accessible to the public. Only basic laboratory equipment and chemicals were used throughout the study, thus making this study economical and applicable in a basic laboratory. The availability of online digital software and databases provide open-source platforms that will ease the efforts in chromosome count.

Keywords: ImageJ; mitotic chromosomes; polyploid; Rubiaceae; single and double staining

Introduction

Neolamarckia cadamba (Roxb.) Bosser (Rubiaceae) is a large, deciduous and fast-growing tree species, and therefore guarantee early economic return within 8 to 10 years (Ho *et al.*, 2014; Pang *et al.*, 2015; Tchin *et al.*, 2018 a,b). It is one of the best sources of raw material for the plywood industry, besides pulp, paper production and medicinal purposes. Phytochemicals can be extracted from different plants to determine its potential (Ianculov *et al.*, 2005; Barbat *et al.*, 2013; Bostan *et al.*, 2013). Therefore, it is a viable option to improve this species by mutation. Improving conventional chromosome count protocol is fundamental in determining the success of mutagenesis through chromosome doubling in this study.

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