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Optimizing flow cytometry analysis for determining ploidy level and genome size of colchicine-induced polyploids of *Neolamarckia cadamba*

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ABSTRACT

Flow cytometry (FCM) analysis plays a crucial role in polyploidization studies. It allows for the rapid identification of induced polyploids or mixoploids and plantlets with unchanged ploidy levels compared to the conventional method of chromosome counting. Therefore, the present study aims to optimize and develop an efficient FCM analysis procedure to determine the ploidy level and DNA content of *Neolamarckia cadamba*. This involved investigating various types of leaf tissues of *N. cadamb*a and lysis buffers to identify the best tissue-buffer combination for FCM analysis. The histograms generated by FCM analysis revealed that only fresh leaves combined with LB01 buffer produced clean histograms with sharper peaks, reduced noise, and low coefficient of variation values. FCM analysis effectively classified the nodal explants of *N. cadamba* treated with colchicine into three distinct groups of polyploid plants: tetraploids, mixoploids, and octoploids. The *N. cadamba* tetraploids were found to have an estimated DNA content of 2.59 ± 0.09 pg, while octoploids showed an increase of DNA content to 5.35 ± 0.24 pg. These results highlight the effectiveness of FCM as a valuable tool in identifying mixoploids among the colchicine-induced polyploids of *N. cadamba*, as compared to the conventional method of chromosome counting. Mixoploids, which are characterized by cells with varying ploidy levels, deserve further investigation in future research.

ARTICLE HIGHLIGHTS

1. Flow cytometry (FCM) analysis is a rapid and efficient method for determining the ploidy level and DNA content in plants.
2. Combining fresh leaves with LB01 buffer produces clean histograms with sharper peaks and reduced noise levels.
3. FCM analysis classified the polyploid plants of *Neolamarckia cadamba* into tetraploids, mixoploids, and octoploids.
4. FCM showed efficacy in identifying mixoploids in *N. cadamba* polyploids compared to traditional methods of chromosome counting.

1. INTRODUCTION

Flow cytometry (FCM) analysis is commonly used to determine the ploidy level, and DNA content of plantlets regenerated from explants treated with varying concentrations and durations of colchicine. FCM plays an essential role in studies related to polyploidization, allowing the distinction between induced polyploids or mixoploids and plantlets with unchanged ploidy levels [1-3]. There are many advantages of using FCM over the chromosome counting method. FCM only uses a small amount of plant tissue, such as the leaf or other non-destructive parts of the plant itself. FCM does not rely on a specific stage of mitosis for analysis, allowing for greater flexibility in sample procurement regardless of harvest time or tissue type [4,5]. Furthermore, the easy and quick sample preparation for FCM enables the bulk screening of many samples in a polyploidization study. Apart from ploidy level determination, total nuclear DNA content (C-value) can also be estimated [6].

The procedure for determining ploidy level and DNA content using FCM involved three main steps: isolation of cell nuclei, fluorochrome staining, and analysis [4]. In the first step, leaf samples are finely chopped using a sharp razor blade in a buffer to release nuclear suspension. The suspension is then filtered through a nylon mesh to separate cell nuclei from debris [7]. It is crucial to use fresh, uncontaminated plant samples for FCM analysis. After filtration, the next step involves DNA staining. Depending on the excitation source of the flow cytometer, one of the following DNA-specific fluorochromes can be used: propidium iodide, ethidium bromide,

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