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Flow cytometry analysis on colchicine induced polyploid of Katokkon peppers (*Capsicum chinense* Jacq.)

Flow cytometry analysis on colchicine induced polyploid of Katokkon peppers (*Capsicum chinense* Jacq.)

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Abstract. A preliminary study was conducted to determine the ploidy level of Katokkon pepper after colchicine induction using flow cytometry analysis. Compared to squash method, flow cytometry analysis can provide effectively faster results of the ploidy levels of the cells. The research was conducted at the Laboratory of Plant Bioscience and Reproduction Biotechnology, Department of Agronomy, Faculty of Agriculture, Hasanuddin University Makassar. Polyploidy of the Katokkon pepper (*Capsicum chinense* Jacq.) were induced by immersing the seeds in colchicine solution at concentrations of 0.00%, 0.0125%, 0.025%, 0.050% (w/v). For each concentration, an immersion time of 1.5, 3.0 and 4.5 hours were employed, respectively. Ploidy level was analyzed using the flow cytometer machine (Partec® Cy-Flow Space). The resulting histogram of the analysis shows differences between control (0.00%) and the rest of Colchicine treatment. Based on the peak position on the histogram, the colchicine concentration and their various immersion time given did not produce tetraploid (4n) plants. Nevertheless, at 0.10% colchicine concentration with all immersion times, it was obtained mixoploid plants with 2n and 4n pairs of chromosomes.

1. Introduction

Polyploidization is one of modern methods in plant breeding to obtain superior plants with better characters by changing the genetic makeup of the plants [1]. Polyploidy plants are attributed to plants that have three or more sets of chromosomes [2]. The polyploid chromosomes represent the number of more than two paired chromosome sets that can be induced chemically using colchicine. Amanah et al. [3] showed in their study that polyploid plants with three and four sets of chromosomes were obtained from the application of colchicine on cayenne pepper (*Capsicum frustescens* L.).

The successful of polyploidization can be determined by observing the number of mutated chromosomes using a simple way such as in squash method or using flow cytometry. Although the squash technique is quite simple, but it does not allow for large sample analysis because it requires a

relatively long time. Compared to the squash technique, which calculates chromosomes with the aid of a microscope [4], flow cytometry is known as a method to rapidly characterize the optical properties of cells and cell components in an individual by measuring the light emitted or scattered from cells or cell components. The method allows a lot of genetic material to be evaluated simultaneously, and the resulting information can be processed quickly. As a result, this technique allows a large-scale comparative analysis to determine polyploid levels [5]. Plant ploidy analysis can be performed quickly and accurately using flow cytometry with the advantages, among others, of ease and speed of sample preparation, as well as allowing analysis of larger numbers of samples [6].

Flow cytometry (FCM) has been widely used to detect polyploidy in plants such as *Catharanthus roseus* L. G. Don [7], *Brassica napus* L. [8], *Cajanus cajan* L. [9], and *Arabidopsis lyrata* [6]. Ploidy from plants is determined by observing data in the form of a peak curve or histogram peak shown on the monitor screen which is obtained based on the glowing rays captured by the detector on the flow cytometer [6]. Examples of flow cytometry results from haploid, diploid, and mixoploid plants is shown in Figure 1.

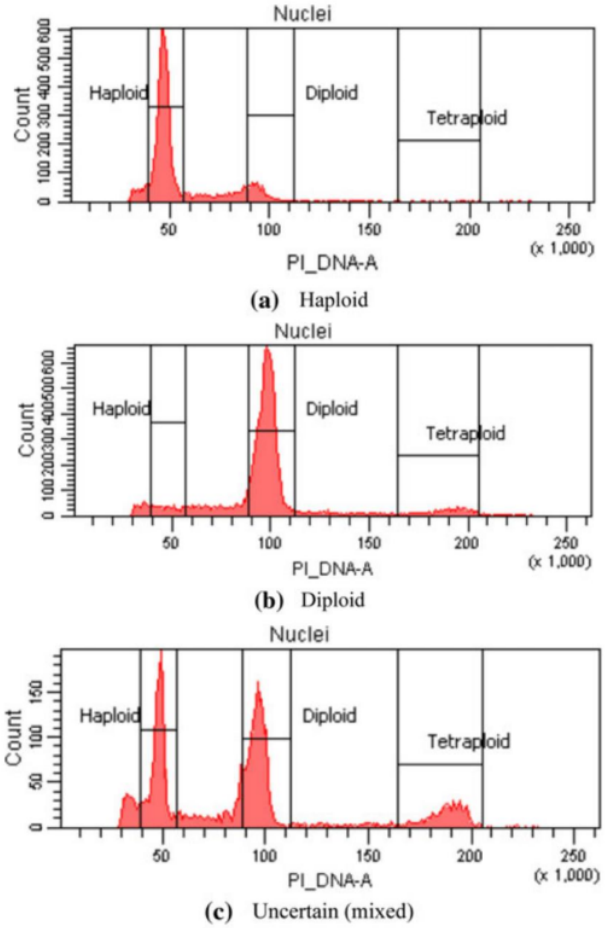


Figure 1. Histogram flow cytometry of *Brassica napus* L. [9].

A preliminary study carried out by the use of high concentrations of colchicine (0.0%, 0.25%, 0.50%, 0.75% and 1.0%) with varied immersion time (1.5 hours, 3 hours and 4.5 hours) in vitro in katokkon pepper (*Capsicum chinense* Jacq.) had been unsuccessful. In this previous study, the results showed that the katokkon shoots failed to develop. The failure was thought due to the colchicine concentration was too high so it was toxic [10]. Therefore, further research was carried out by lowering the colchicine concentration to get the best concentration that could produce tetraploid plants based on plant morphological characters in culture bottles and the results of ploidy level analysis using flow cytometry.

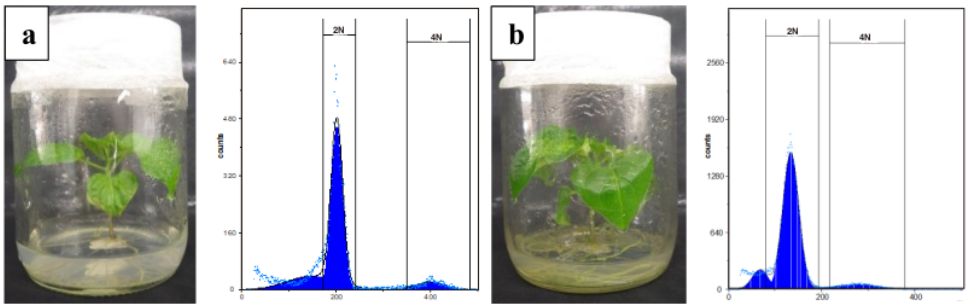
2. Methodology

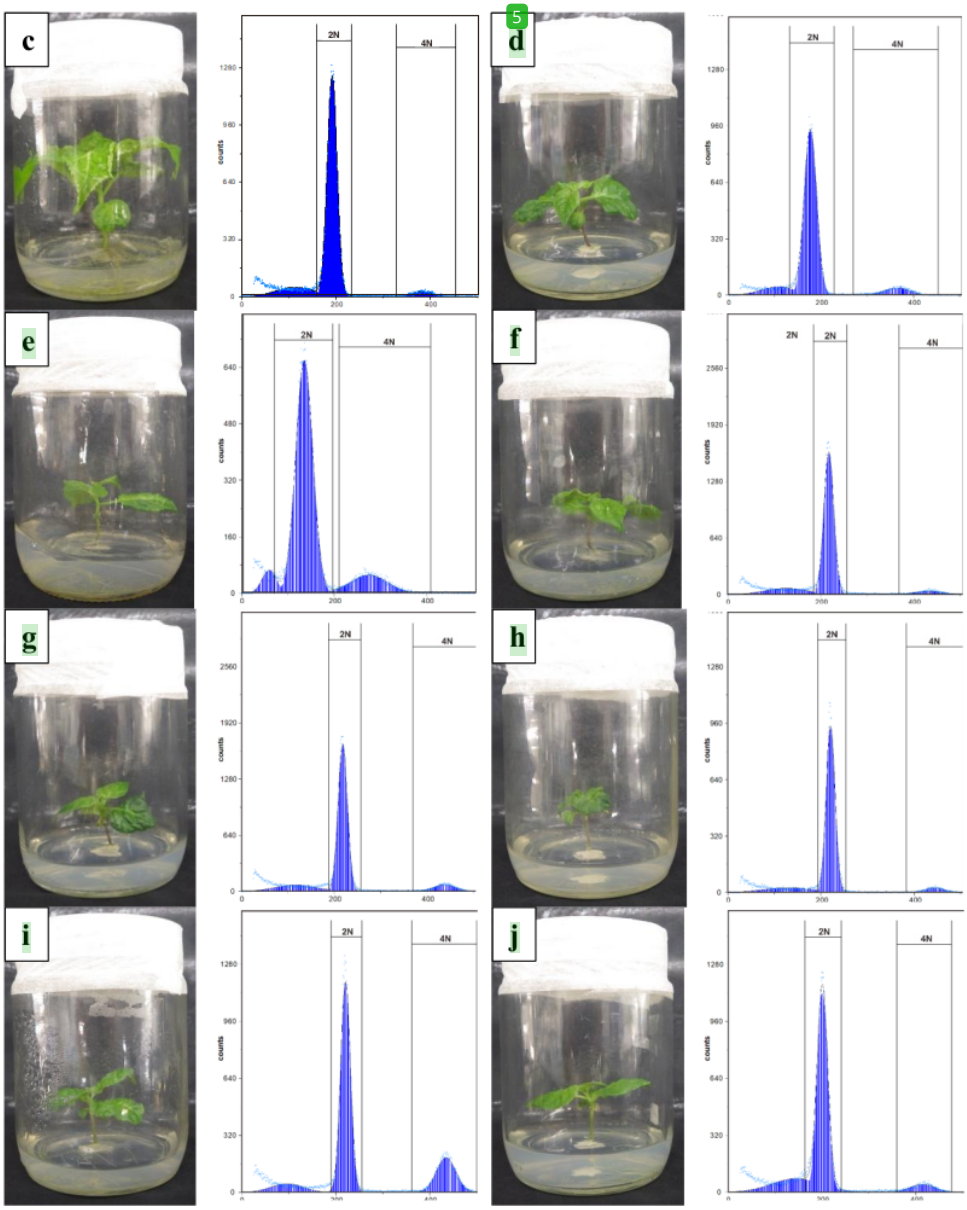
The research was conducted at the Laboratory of Plant Bioscience and Reproduction Biotechnology, Facuty of Agriculture, Hasanuddin University, Makassar. The method used in planting katokkon pepper seeds (*Capsicum chinense* Jacq.) and colchicine induction is based on the results of preliminary study that have been conducted [10]. Lower concentration of colchicine (0.00%, 0.0125%, 0.025%, 0.050% and 0.10%) and immersion time of 1.5, 3.0 and 4.5 hours were used in the recent study compared to those used in the preliminary studies.

Analysis of the degree of ploidy was carried out using the Partec® Cy-Flow Space flow cytometer. Young leaves sample with a size of 0.5 x 0.5 cm was placed separately in a Petri dishes. The leaves were given 250 µl of Cystain Pi extracting buffer. Leaves chopped with a razor blade until smooth for ± 60 seconds. The extract was then filtered on a sample tube filter to obtain about 0.2 µl of phytrate. A total of 800 µl of propidium iodide dye was inserted into the sample tube. Then, the sample tube was placed into the FCM machine for analysis and the results can be obtained directly observed on a computer screen in the form of a histogram.

3. Results and discussion

Colchicine induction is a mechanism that is often used to encourage mutations, resulting in changes in the shape, size and number of chromosomes. In this study, the changes that occurred were marked visually by the size of the plantlets and histograms resulting from the flow cytometry analysis. Colchicine concentration has a significant effect on katokkon pepper (*Capsicum chinense* Jacq.) When viewed from the plantlet morphological characters (Figure 2). Colchicine-induced plantlets were characterized with shorter plants, narrower leaf width and fewer leaves than controls. Polyploid individuals have different morphological characters from diploid plants [3]. Plants treated with colchicine would have shorter plants than diploid plants as control [11].

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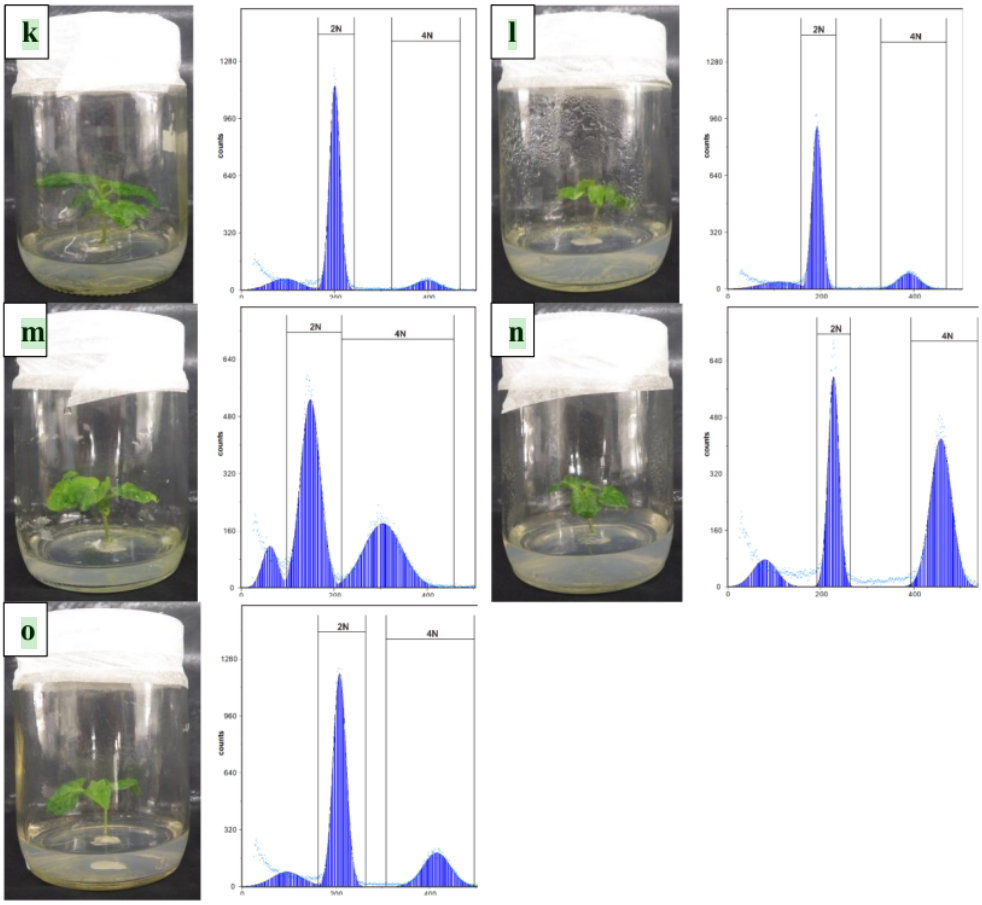

Figure 2. Katokkon pepper plantlets (*Capsicum chinense* Jacq.) treated with several colchicine concentration and immersion time, and the flow cytometric histogram : a (0.00% colchicine, 1.5 hours), b (0.00% colchicine, 3.0 hours),c (0.00% colchicine, 4.5 hours), d (0.0125% colchicine, 1.5 hours), e (0.0125% colchicine, 3 hours), f (0.0125% colchicine, 4.5 hours), g (0.025% colchicine, 1.5 hours), h (0.025% colchicine, 3 hours), i (0.025% colchicine, 4.5 hours), j (0.05% colchicine, 1.5 hours), k (0.05% colchicine, 3 hours), l (0.05% colchicine, 4.5 hours), m (0.10% colchicine, 1.5 hours), n (0.10% colchicine, 3 hours), o (0.10% colchicine, 4.5 hours).

The histogram of the analysis using Partec® Cy-Flow Space shows the difference between the control and the colchicine treatment results. Ploidy from plants is determined by observing data in the form of a peak curve or histogram peak shown on the monitor screen which is obtained based on the glowing rays captured by the detector on the flow cytometer [6]. The peak on channel 200 shows diploid plants while on channel 400 shows tetraploid plants. Based on the peak position on the histogram, the colchicine concentration and immersion time given did not produce tetraploid plants. However, at 0.1% colchicine concentration with various immersion times, mixoploid plants were obtained, this can be seen from the results of the histogram which showed a high enough peak on channel 400 (Figure 2: m, n, and o). The peaks seen on channels 200 and 400 at 0.1% colchicine concentration indicate that the plantlets are mixoploid plants with two and four pairs of chromosomes.

4. Conclusion

Use of the flow cytometer in the analysis of polyploidization of colchicine induced katokkon pepper using a colchicine concentration of 0.0125% - 0.1% with 1.5 - 4.5 hours of immersion, respectively resulted in mixoploid plants with 2 and 4 pairs of chromosomes.

Acknowledgment

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