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Application of flow cytometry in plant science

Deniz Aksoy *[1], **Furkan Ayaz** [2]

[1]*Alata Horticultural Research Institute, Türkiye, denizsanal06@gmail.com*
[2]*Mersin University, Biotechnology Research and Application Center, Türkiye, furkanayaz@mersin.edu.tr*

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Abstract

Chromosomal determinations have been used in the identification of many diseases and anomalies. In the first studies, the biochemical and physical properties of cells were determined using a primary microscope. Flow cytometry technique has become an important part of modern plant breeding programs as conventional chromosome counting methods via microscope etc. become insufficient. Flow cytometry is an application in which the properties of biological particles, especially cells, are measured in a liquid stream. Flow cytometry has been widely used in plant science since the 2000s due to its fast setup, quick sample preparation and reading of thousands of particles per second. In this proceeding we will discuss the flow cytometry applications in plant science studies.

Introduction

Chromosome measurements first gained importance in human health research. Chromosomal determinations have been used in the identification of many diseases and anomalies. In the first studies, the biochemical and physical properties of cells were determined using a primary microscope. Clinical use of chromosome measurements has expanded further ın recent years and started to be used frequently in microbiology, plant and animal sciences [1]. Changes in the number of chromosomes in living organisms are called "ploidy", and the presence of more than two sets of chromosomes in somatic cells is called "polyploidy". Polyploidy is an increase in the number of all chromosomes in a plant's genome at the same rate. An issue where the ploidy level gains importance is plant breeding studies. Knowledge of plant DNA contents has become essential for many plant breeding programs or techniques [2]. One of the biggest problems encountered in plant breeding is the narrowing and insufficiency of genetic variation over the years [3]. Especially in crossbreeding breeding programs, wild and semi-wild plant forms with genetic variation are used [4]. It is important to determine the cytogenetic information of wild forms.

Flow cytometry technique has become an important part of modern plant breeding programs as conventional chromosome counting methods take a lot of time and become insufficient. Information on the DNA content of plants has increased over time. This has led to the emergence of breeding programs that directly target ploidy changes, called 'Ploidy breeding'. Polyploidization in plants often results in increased cell size leading to positive morphological changes. Organs of polyploid plants such as stems, leaves and flowers are larger than diploid ones and their surface areas are wider. Since these plants have larger cells and a higher amount of chlorophyll, they attract attention with their dark green color. Their photosynthetic potential is also higher than diploids. It is also estimated that there is a positive correlation between genome size and ploidy level [5,6]. Flow cytometry is a very useful tool for identifying DNA contents in plant cross-breeding.

Results

Flow cytometry is essentially both a technique and a device. It is technically the measurement of the properties of cells or particles when they are in a flowing fluid. With this method, the structural features and DNA amounts of cells can be determined. When evaluated within the scope of the device, it is a machine that creates usable information by transmitting different wavelengths formed by the laser radiation of the device to the system through fluorescent dyes that are attached to the structure of DNA. Propidium iodide, ethidium bromide and acridine orange fluorescent dyes are mainly used in flow cytometry.

Cells go through a series of stages known as cell cycle. Flow cytometry technique which is developed for plant research basically uses this cycle of grow. With Flow, it is possible to determine the number of cells in which division phase of the cells. In this way, information about the proliferation rate of cells is collected. During the process of device while diploid sample cells are in G0/G1 phase, DNA content of cells in S (synthesis) phase will be between diploid and tetraploid cells. Cells in G2 and Mitosis will be viewed as tetraploid since they carry 4n DNA.

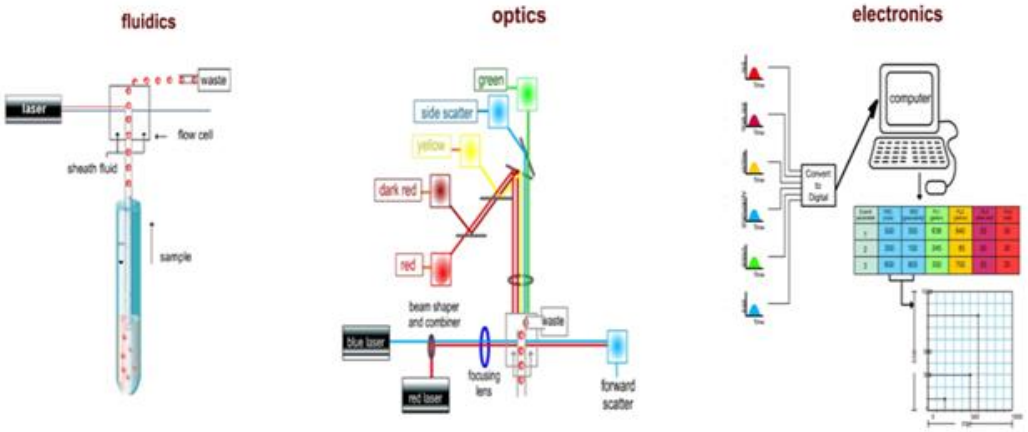

Figure 1. Components of flow cytometry device [9]

Flow cytometry consists of 3 basic steps; fluidics, optics and electronics. The sample solution, which is put through the liquid flow system, moves in front of the laser in a single line through the liquid called sheath fluid, which surrounds it and does not mix with the sample solution. While passing in front of the laser, the beam hitting the particles is dispersed at different wavelengths and this fluorescent dispersion is collected by filters and transmitted to the photodetector. The optical signals from the detectors are converted into electrical signals with Photomultiplier tubes and finally transferred to the computer [7,8].

Conclusion

Although the flow cytometer setup costs are high, the operating and maintenance costs are low. Correct calibration is required. Otherwise, it is possible to measure all structures other than the particles desired to be measured. Flow cytometry has been widely used in plant science since the 2000s due to its fast setup, quick sample preparation and reading of thousands of particles per second [5, 6].

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