Application Note

DEPArray™ Rare Cell Isolation Technology

Morphological Evaluation and Targeted Isolation of Plant Cells and Pollen Grains

Introduction

The agronomic industry is the oldest and largest industry using biotechnological processes to advance and improve products. Several improvements that have been introduced in the field of plant biology by the newest characterization and isolation approaches are a harbinger of future contributions that will arise from improvements in cell culture techniques and genetic or germ line selection for crops. The development and application of systems specifically focused on improving the starting materials of plant products, like seeds and tissue culture products, are still needed and could save a lot of resources and improve productivity in the industry. Here we investigated the possibility to morphologically characterize and isolate single and pure Plant Cells and Pollen Grains from heterogeneous samples with DEPArray™ technology. In fact, the DEPArray™ system is distinguished from other technologies because is generally applicable to all vegetative cells that can be morphologically identified, does not rely on the availability of specific biomarkers and enables plant biologists to quickly and easily isolate discrete cell population in a routine manner. In addition, we show that the selected cells remain viable after analysis and selection, demonstrating the compatibility of DEPArray™ technology with these biological samples.

Materials and Methods

The Plant Cell and Pollen Grain suspensions were kindly obtained from Dr. Arie Draaijer (Fytagoras BV – Leiden, NL). A small amount of the two cell suspensions, estimated to be a few thousand cells, were respectively loaded into the DEPArray™ cartridge for analysis using Silicon Biosystems' standard procedure. We obtained digital image galleries of the cells relying on plant tissue auto-fluorescence (using FITC channel) and the standard bright field scan. Shape, size and brightness measurements were performed with the integrated DEPArray™ instrument analysis software enabling population analysis from scatter plots as well as image evaluation. A targeted subpopulation was also selected for output viability evaluation using fluorescein diacetate (FDA) hydrolysis assay.

Results

The DEPArray™ technology was able to trap, analyze, route and recover a wide range of cells (from 25 to 40 µm):

- the scatter-plot and image galleries generated by analysis on the DEPArray™ instrument clearly showed the possibility to selectively identify and classify different populations in Plant Cell or Pollen Grain samples using fluorescence intensity and images inspection characteristics (**fig.1**).

- the design of random multi-recoveries experiments allowed the analysis of cell viability ratios in each cell population before and after manipulation with the DEPArray™ system. We counted as vital just the cells that actively converted the non-fluorescent FDA into the green fluorescent compound "fluorescein" (**fig.2**), obtaining, as mean of three different experiments, 95,0% ± 4,6 of viable cells (**tab.1**).

Finally, pure recoveries of plant cells or pollen grains were carried out successfully (**fig.3**).

Conclusions

Manipulation and fine selection of plant cells have unique challenges and methodologies. Improvements are needed in the characterization, safety, quality control of food materials and processing methods. Fortunately, methods have been developed to overcome these challenges, and successful imaging of plant cells and tissues is fully possible with the right tool. The DEPArray™ technology joins this venture thanks to its ability to characterize different-sized cell populations and isolate pure single cell. Furthermore, automation with DEPArray™ technology could significantly reduce laboratory costs and time, maintaining a high level of standardization and reliability in crop's varieties selection.

Keywords: DEPArray™; plant cells; pollen grains; seed; pure cells

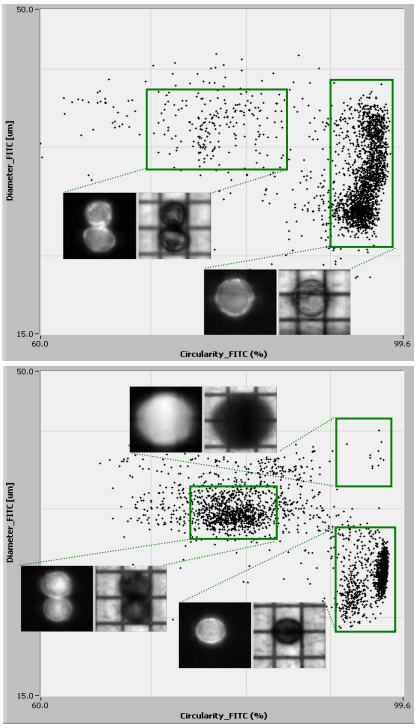

fig.1 A DEPArray™ system scatter plot allowed identification of well-defined populations of Plant Cells (upper panel) and Pollen Grains (bottom panel). Thanks to the technology it is possible to gate cells with different properties and operator-mediated inspection could refine the final selection. Representative image gallery showing various "shape and size" cells identified in Plant cells and Pollen Grains subpopulation. Images were presented in Full Dynamic view.

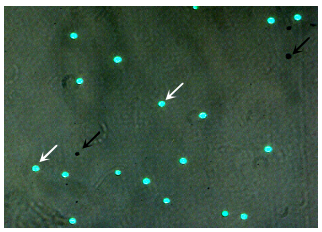

fig.2 Representative field of merged FITC and BRIGHTFIELD channels obtained from DEPArray™-isolated plant cells. Fluorescein-positive cells (white arrows) were counted as viable, while fluorescein-negative (black arrow) were evaluated as dead cells.

	Input Viability	Output Viability	O/I Viability
1st Exp	63,1%	60,4%	95,7%
2nd Exp	51,8%	51,4%	99,3%
3rd Exp	51,8%	46,6%	90,1%
		mean	95,0%
		std dev	4,6%

tab.1 Detailed results of measured Input/Output viability ratio obtained in three different experiments.

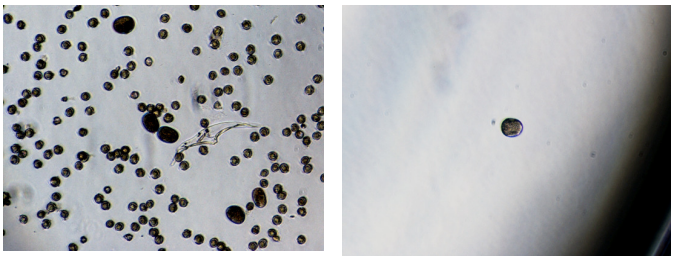

fig.3 DEPArray™ technology allowed recovery of targeted cells as individual pure single-cells. Representative field of heterogeneous sample (left) and pure single-cell (right), before and after DEPArray™ system selection.

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