APPLICATION REVIEW

Pollen Viability

The determination of pollen quality is essential in the creation of varieties and for the production of seed. However, determining the viability or the developmental stage of pollen grains used to be a challenge.

So far, the methods used (microscopy counting with labels) were time consuming and cumbersome. A new technology, developed by Amphasys, enables automated analyses based on the analysis of isolated cells by impedance flow cytometry.

Let's take a closer look at the advantages this technology provides to Vegenov, a resource centre specializing in plant research. The following article was published on Vegenov's Blog.

About Vegenov

Vegenov is a non-profit organization specialized in plant science applied research. They offer transparent and confidential support for your processes.

Highly qualified in molecular and cell biology, plant protection and nutrition, sensory and nutritional quality; they rapidly provide you with quality custom R&D solutions.

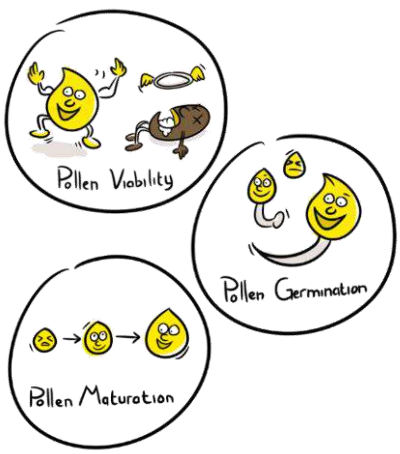
Why Measuring Pollen Quality

The storage of mature pollen grains over time makes it possible to carry out hybridizations between genotypes that do not flower at the same time.

In seed production, the quality of pollen and the impact of environmental conditions on flowering are paramount. Male sterility is sought to simplify the production of F1 hybrid seeds.

In the laboratory, the use of androgenesis requires the collection of immature pollen, i.e. microspores, at certain precise stages to produce doubled haploid lines.

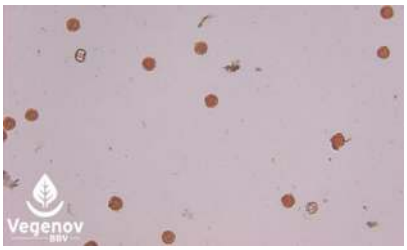
A reliable indication of pollen quality parameters such as developmental stage, viability and germination capacity is therefore crucial to the success of all these processes.



Current Methods are Cumbersome and Unrealiable

Pollen is a type of cell with a very specialized development in the plant.

During its maturation, it will change size, the composition of its cytoplasm will evolve and it will undergo cellular divisions. A very strong cell wall develops that will protect it from dehydration and threats of the external environment. Every plant species has unique pollen, and until now the analysis methods were different every time.



Carmine acetic staining of viable cells.

Methods commonly used to determine the quality of pollen are:

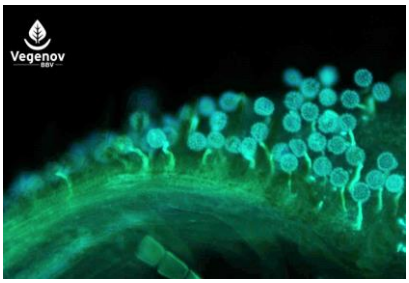
- Staining and counting by microscopy to determine developmental stage or viability
- *In vitro* or *in situ* germination of pollen

These methods have multiple constraints. Pollen viability stains differ among species (Jahier *et al.*, 1992) and are usually difficult to interpret. Moreover, microscopy does not allow counting of large quantities of cells. The counting takes long and is tedious. This leads to low statistical relevance prone to errors.

FDA staining allows measurement of pollen viability, here eggplant. Cells with high green fluorescence intensity are considered viable.

In vitro pollen germination tests involve complex and varied culture media that depend on the species. This often leads to problems with the analysis. For some species, no protocol could be developed (e.g. cereals).

In situ germination observation methods have been developed and generally work well, but are very cumbersome to implement: a few hours after pollination, the fertile parts of the flowers are taken from the plant and fixed. The samples are then individually stained and observed under a microscope (Jahier *et al.*, 1992).



Germination of pollen grains on the stigma as observed under a fluorescence microscope after staining with aniline blue.

Innovative Single Cell Analysis by Impedance Flow Cytometry

A method for performing these different types of analysis in a single measurement has been developed by Amphasys. It is based on impedance flow cytometry analysis of single cells.

When applying an alternating electric current, the impedance of each cell is measured in a microfluidic chip. The impedance of a cell varies according to:

- The frequency of the alternating current
- The capacitance of the membrane
- The volume of the cell
- The conductivity of the cytoplasm

The Ampha Z32 Impedance Flow Cytometer set up in the Lab with the autosampler and a laptop showing measurement results.

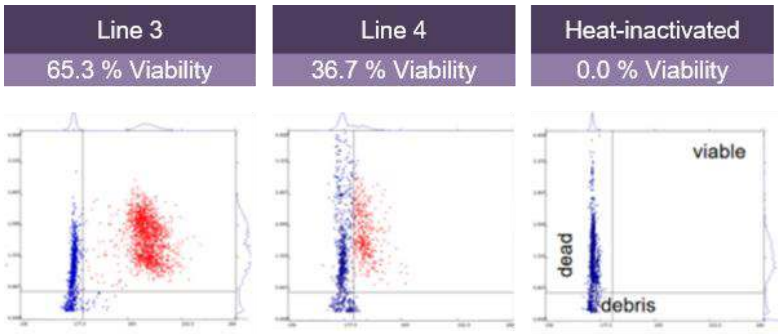


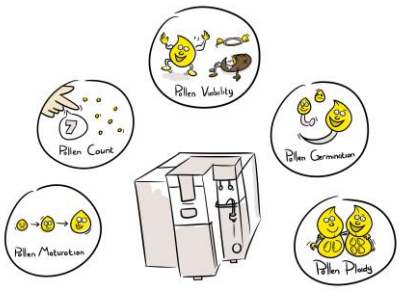
Figure left: A set of individual impedance measurements made on pollen samples from two wheat varieties. The impedance of each cell is represented by two parameters reported on the horizontal and vertical axes. The cloud of blue dots represents the dead cells (identical to the inactivated pollen control) and the red cloud the living cells. The percentage of viable cells is calculated automatically.

This technique makes it possible to automate the counting of pollen grains and to measure their viability, which varies according to, for example, collection conditions, genotypes, the impact of temperature or insecticide treatments. It is non-destructive, label-free and does not require the use of optical tools. Automating the counting and measuring of pollen viability makes this step much less cumbersome and several ten-thousand cells can be analyzed, instead of around a few hundred cells by microscopy.

Measure More

The Ampha Z32 instrument is easy to transport and can be powered by a battery which allows to measure in the field if necessary.

Depending on the plant species, and following preliminary optimization, it is possible to carry out even more precise analyses. This technique allows to monitor pollen developmental stages (e.g. detection of the stage of abortion of sterile varieties), to measure the pollen germination potential and even to differentiate cells according to their ploidy level (Heidmann *et al*., 2016).



Vegenov has been using this technology for more than a year to perform different types of analysis for its clientele of breeders, seed companies or seed producers.

Murielle Philippot, R&D engineer specialized in haplomethods in the cell biology team at Vegenov demonstrates the advantages of using this new technique:

«Thanks to the tool of Amphasys, we were quickly able to optimize and measure pollen development in numerous plant species. By measuring impedance, we can follow the different developmental stages and the viability of important cell populations in a very quick and reproducible way.

Before, we did our analyses by staining and counting under the microscope. Obtaining this new technology allowed us to multiply the number of analyses, which multiplied the reliability of our results. We also started to optimize the analysis of microspore cultures, to accurately characterize and follow their development during androgenesis.»

Murielle Philippot at Vegenov

References:

Original article: blog.vegenov.com/2018/02/mon-pollen-est-il-vivant/

Heidmann I., Schade-Kampmann G., Lambalk J., Ottiger M., Di Berardino M. (2016) Impedance Flow Cytometry: A Novel Technique in Pollen Analysis. PLOS ONE 11(11): e0165531

Jahier J. (1992) Techniques de cytogénétique végétale. Editions Quae 183 pages

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