Amphasys Customer Application Story —

Bayer CropScience





Thousands of pollen viability measurements provide a statistically relevant data base for line selection

In wheat breeding, as with many other crops, pollen viability overall and over time, total number of pollen shed and the period of blooming are very important elements. Pollen viability is an essential factor for the selection of lines in R&D of seed production. Until now, pollen analysis was very limited with respect to the number of samples that could be processed and the reliability of the result. Staining methods are bound to time-consuming laboratory work, hence transportation of the biological material in question is needed and no fast and reliable in-field method was available. This changed when Dr. Michael Schmolke from Bayer CropScience became aware of the IFC (Impedance Flow Cytometry) technology of Amphasys. This label-free method with easy sample preparation and fast in-field measurement was the key solution to analyze thousands of samples during the short production season.

Introduction

"A meticulous planning of the setup of this large in-field campaign over four weeks, covering several thousand measurements, was crucial for success" says Dr. Schmolke. "This included the selection of the anthers with the desired stage of development, the sample preparation process with the appropriate choice of buffers, the mechanical pollen extraction, shaking and filtering, as well as the recording of the exact location in the field where the sample was collected and the environmental conditions like weather and temperature" he explains. "Our sample container of choice was a 5ml safelock reaction tube with 2ml of buffer prefilled. This allowed a very efficient sample collection in the field", Dr. Schmolke concludes.



In-field setup of the campaign

The Ampha Z30 instrument was placed in a large van and was powered by a 12V car battery adapter. "We would choose the newly available battery pack today" states Dr. Schmolke. "The battery would allow a continuous operation without the need of a car." The sample tubes were dutifully labelled and prefilled with 2ml of AmphaFluid No.6 in the morning, before leaving for the fields. Once there, part of the team went into the field with the tubes and collected the anthers from the respective plants and brought them back to the car within minutes. The other part of the team took care of the mechanical extraction of the pollen and the measurement. Dr. Schmolke summarizes that "over 100 samples could be processed this way in less than four hours in the field. The use of the equipment in the field also guaranteed immediate measurement after collection, free of aging effect on the wheat."

Data analysis and results

All the samples measured in the field were analyzed at a later point in time. A crucial part however was the correct population gating to discriminate viable from dead pollen. A control measurement of dead pollen was used to define the separation line between active and inactive pollen. The results show viability in percent of the overall population and in effective counts of cells (pollen). "Our main interest was in the viability of the pollen to compare lines. The set of data allowed a comparison of pollen viability between wheat lines and a selection of the lines to proceed with in R&D. It allowed data processing in a fast and reliable way" confirms Dr. Schmolke very satisfied with his campaign.



Amphasys AG

Amphasys AG is a Swiss based innovator in the field of flow cytometry. The label-free Impedance Flow Cytometry (IFC) allows fast, reliable and accurate detection of cell viability, microspore development stages, ploidy and other relevant factors in research and production of seeds and fruits.

We appreciate the use and valuation of our technology by Bayer CropScience in Gatersleben, and we especially thank Dr. Michael Schmolke for his kind support for this testimony.



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