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Label-free Pollen Viability Determination by Impedance Flow Cytometry

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Abstract

Analysis of pollen **viability** plays an important role in various aspects of plant breeding and production processes. Here, we show that the label-free analysis of pollen based on their dielectric properties by impedance flow cytometry (IFC) is a very efficient method for the determination of pollen viability and microspore **maturation** in a high throughput manner. In addition, we demonstrate the unique potential of IFC in pollen analysis with respect to **germinability** and **ploidy**.

Background

Classical methods like staining techniques or in vitro germination assays are connected with disadvantages due to laborious preparation steps, fading of fluorescent dyes and low reproducibility. IFC overcomes these limitations thanks to a high-speed microfluidic approach purely based on the electrical properties of multi-dimensional high frequency impedance. Within seconds, thousands of pollen grains are individually analyzed in a microfluidic “lab-on-chip” allowing detailed cell characterizations.

Method

Figure 1: Pollen viability readings are obtained in three simple steps: (i) sample preparation, (ii) measurement, (iii) analysis of the results. The sample is prepared by re-suspending pollen in analysis buffer. After a subsequent filtration step the sample is automatically pumped through the microfluidic analysis chip. The associated software reports and visualizes the results.

Results

Viability analysis via IFC differentiates between dead and viable pollen

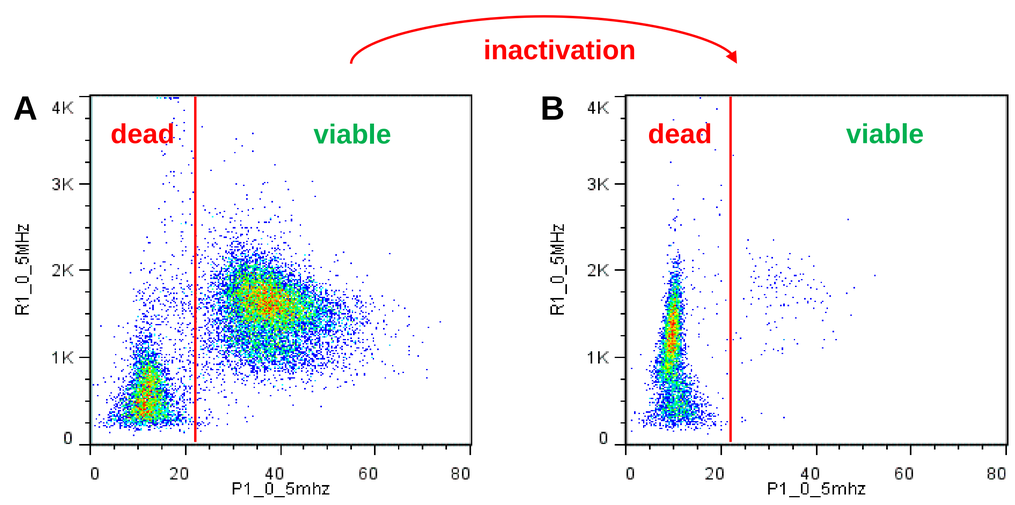

Figure 2: Differentiation between dead and viable sweet pepper pollen by IFC. A, dead (left) and viable (right) population of freshly harvested pollen; B, the same pollen population after inactivation.

Determination of developmental stages and viability of pollen

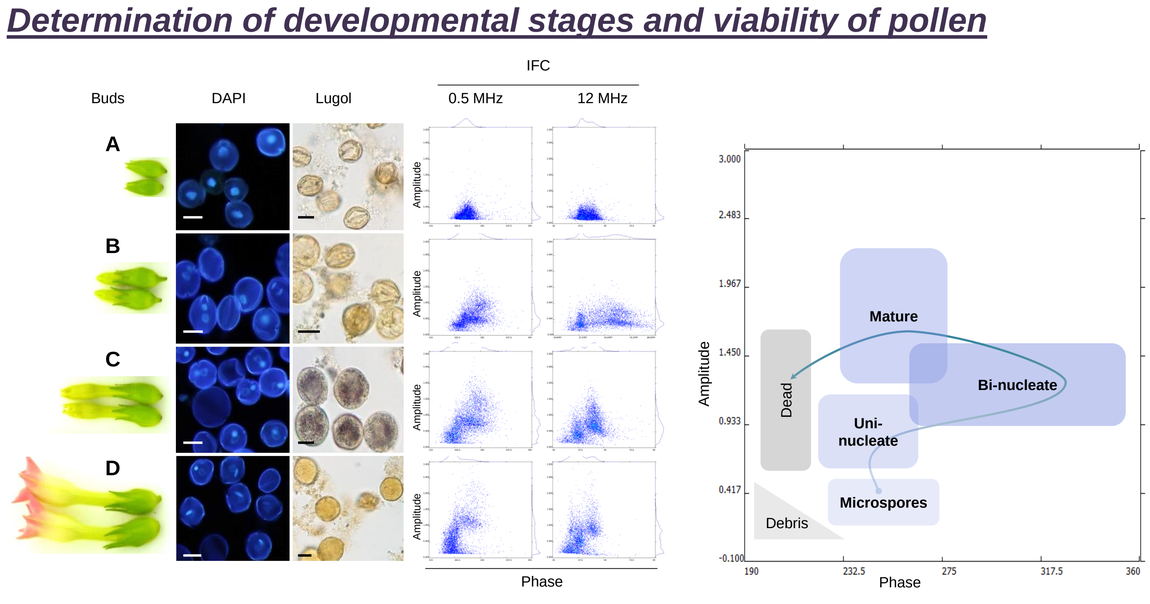

Figure 3: Developmental stages of tobacco pollen according to flower bud size (Buds) was followed by stage determination (DAPI), starch accumulation (Lugol) and IFC analysis at 0.5 and 12 MHz. The stages (A-D) represent A: uni-nucleate, B: bi-nucleate, C: late bi-nucleate, D: mature pollen grains. Scale bars: 20 µm

Combined analysis of ploidy and viability by IFC

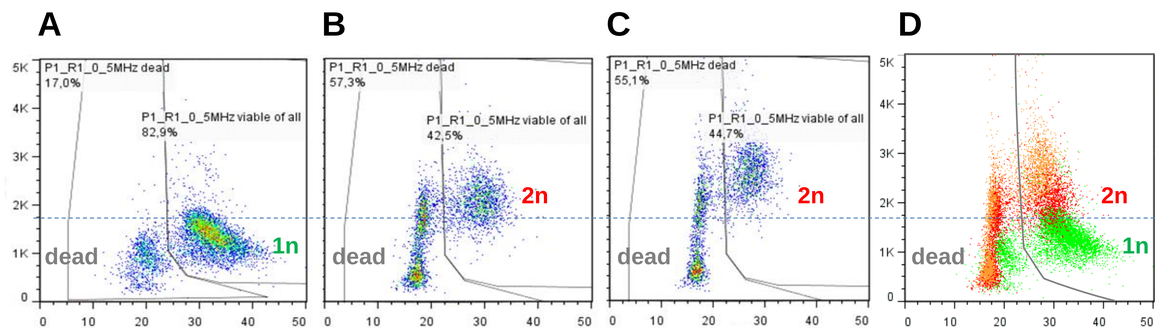

Figure 4: IFC based analysis of ploidy and viability. A, haploid tomato pollen; B and C, diploid tomato pollen; D, overlay of A (green), B and C (red and orange).

References

Heidmann et al. 2016. Impedance flow cytometry: A novel technique in pollen analysis. PLOS ONE **11**(11): e0165531.

Crocetti et al., 2014. Impedance flow cytometry gauges proliferative capacity by detecting TRPC1 expression. Cytometry A **85**: 525-536.

David et al., 2012. Viability and membrane potential analysis of *Bacillus megaterium* cells by impedance flow cytometry. Biotechnology and Bioengineering **109**: 483-492.

Schade-Kampmann et al., 2008. On-chip non-invasive and label-free cell discrimination by impedance spectroscopy. Cell Proliferation **41**: 830-840.

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