Impedance Flow Cytometry
A novel method for pollen viability determination

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Background

Analysis of pollen viability plays an important role at various aspects of plant breeding and plant production processes. Pollen viability is generally determined by various classical methods like staining techniques or in vitro germination assays. The disadvantage of the numerous staining techniques is that they have to be adapted per species and the resulting data do not always correlate with in vitro germination. Both, the current methods analysing pollen viability and germination are limited in the number of cells that can be analysed in a certain time frame and they are laborious in preparation and analysis. Here, we present a novel, label-free approach for the determination of pollen viability and maturation grade based on high-throughput single cell analysis by impedance flow cytometry (IFC). The technique is based on an improved Coulter counter which analyses individual pollen grains via a microfluidic chip and permits impedance measurements in a broad radiofrequency (RF) range (0.1 – 30 MHz), allowing detailed cell characterisations.

Method

Viability analysis via IFC differentiates between dead and viable pollen

Results

Determination of developmental stages and viability of pollen

Combined analysis of ploidy and viability by IFC

Conclusion

Impedance flow cytometry

- Is a reliable and efficient method to analyse pollen viability
- Is independent of any staining procedure
- Allows the determination of developmental stages of pollen and size-based ploidy analysis
- Can be applied to all species also in a non-lab environment

References


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Figure 1: The Technology. In an electric alternate current (AC) field cells have specific, frequency-dependent dielectric properties that can be visualised by IFC. Low frequencies (< 0.5 MHz), for example, reveal the size of cells. Higher frequencies (0.5 to 6 MHz) provide information on the membrane capacitance and frequencies above 4 MHz information about the cytoplasmic conductivity.

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.

Figure 3: Developmental stages of Brassica pollen. A, an inflorescence of Brassica sp.; B, Scheme of pollen development from uni-nucleate to tri-cellular pollen, n = vegetative nucleus, g = generative nucleus; C, live images of microspores/pollen; D, DAPI staining of corresponding stages; E, IFC analysis on single bud level.

Figure 4: Pollen size based analysis of ploidy and viability. A, haploid tomato pollen; B and C, diploid tomato pollen; D, overlay of A-C.