APPLICATION NOTE

TOMATO
Pollen Germination vs. Pollen Viability

The ability of a pollen grain to fertilize a female egg is an essential step in plant reproduction. While *in-vitro* germination is often regarded as the gold standard method for assessing pollen viability it comes with significant drawbacks when compared to other methods. In this application note *in-vitro* germination and IFC are compared.

Conclusions

- IFC and germination show a good correlation when the correct pollen sample preparation and germination conditions are applied.
- IFC method is more accurate, less prone to bias and significantly faster. For the analysis of 100 samples a time savings of at least 10 hours is achieved.
- For optimal results, a controlled rehydration process of 30 min needs to be applied.

Results

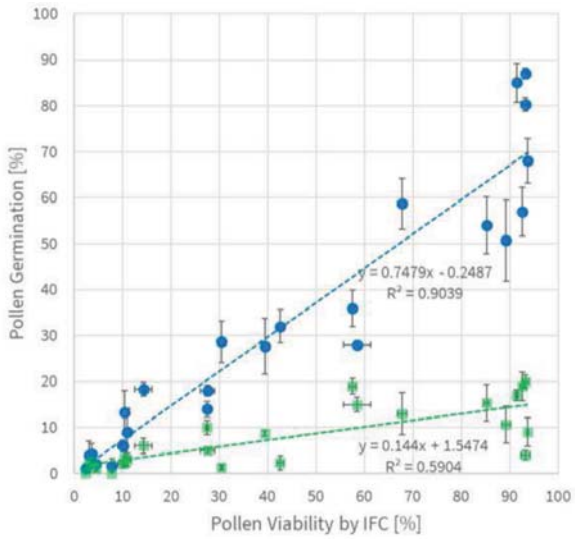
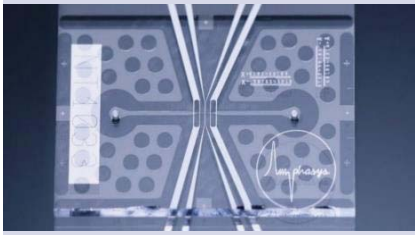


Figure 1 underlines the importance of the germination buffer composition and how strongly it affects the germination results. For pollen with a viability of more than 80%, germination rates of less than 20% were obtained with the standard agar germination buffer. Using an optimized germination buffer the germination rates reached similar values as obtained with IFC.

Ampha Z32
Impedance Flow Cytometer

- Rapid
- Accurate
- Reproducible
- Label-free
- Portable for on-site analysis



Lab-on-a-chip technology

- Small samples required
- Single cell analysis (no average values)
- Statistically large sample sizes
- Sensitivity and throughput tunable by chip choice

Figure 1: Correlation between viability determined by IFC and germination. Germination was measured using a standard germination agar medium (■) and a modified liquid germination buffer (●). Dashed blue and green lines represent the trend lines with the corresponding equations and correlation coefficients. Y-error bars indicate the standard deviation of the germination, x-error bars the ones of the IFC viability method (normally smaller than dot size).

For germination, triplicates of 100 cells were counted which took about 10 minutes for an experienced person. The corresponding standard deviation was about 10%. For IFC duplicate measurements of 20'000 cells were analyzed automatically within 1 minute with a standard deviation of less than 1%.

As shown in Figure 2, rehydration of dry tomato pollen does not necessarily occur in a liquid medium. Rather, pollen grains need a humid environment to properly restore the membrane integrity and thus their viability, an observation previously described by Shivanna and Heslop-Harrison [5].

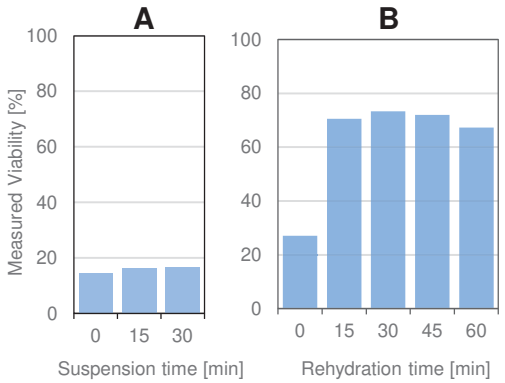


Figure 2: Effects of various treatments on the viability measured by IFC. A dehydrated and stored tomato pollen sample was suspended in AF6 buffer and its viability directly measured by IFC after the indicated incubation time (A). Alternatively, a pollen sample was first rehydrated for up to 60 min before analysis by IFC (B).

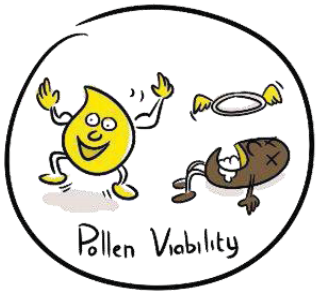
Discussion

As described earlier [1], viability is in general slightly higher than *in vitro* germination. This could be due to a systematic overestimation of viability or a systematic underestimation of *in vitro* germination when compared to *in vivo* germination.

The primary cause for loss of the ability to germinate seems to reside in an irreversible damage of the pollen membrane integrity [1]. Membrane integrity can easily be assessed by impedance flow cytometry (IFC) and has been shown to be a reliable measure for cell viability [2].

It was reported that pollen viability measured by IFC or staining methods shows somewhat higher values than those obtained by *in-vitro* germination assays. There are several reasons that explain this observation:

- Germination is a lengthy process that can take several hours such that the result depends on the incubation time.
- The germination medium has a pronounced impact on the rate and capacity of germination.
- Stored pollen are often dehydrated. Lack of appropriate rehydration prior to *in-vitro* germination assays in general leads to slower or incomplete germination.



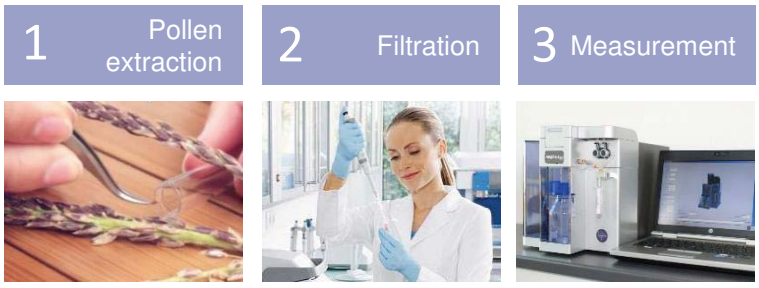
Materials and Methods

Tomato pollen (*Solanum lycopersicum)* stored at -20°C was rehydrated for 30 min at room temperature (RT) in a humid atmosphere as further described in the corresponding Quick Guide [3]. For *in-vitro* germination two different assay media were used in 12-well culture plates: (a) Agar medium containing 0.6% agar in a modified germination medium according to Brewbaker and Kwack [4] and (b) liquid medium developed by Amphasys containing the same minerals as in (a), but with a reduced osmolarity.

A small amount of rehydrated pollen was incubated in these media for 2 hours at RT and the pollen grains were regarded as germinated if the formed pollen tubes were longer than the double of their diameter. IFC viability was measured with the standard operational procedure described by Amphasys.

Tomato pollen (Solanum lycopersicum)

Rapid 3-Step Workflow



The Ampha Z32 Pollen Analyzer

Compared to traditional pollen staining or germination protocols, which require sample incubation and tedious counting of cells under a microscope, the Ampha Z32 Pollen Analyzer offers:

- Simple sample preparation – no staining, media preparation or incubation is required
- Fast results – with measurements that take less than a minute
- One method for all species – this technology works with over 200 species

REFERENCES

[1] Shivanna K. R. (2003). Pollen Biology and Biotechnology. CRC Press, Taylor & Francis Group, Boca Raton (Florida, USA).
[2] Heidmann I. *et al.* (2016). Impedance flow cytometry: a novel technique in pollen analysis. PLoS One 11:e0165531.
[3] Quick guides: amphasys.com/amphasys-downloads/#quick_guides
[4] Brewbaker J. L. and Kwack B.H. (1963). The essential role of calcium ion in pollen germination and pollen tube growth. American journal of botany, 50 (9), 859-865.
[5] Shivanna K. R. and Heslop-Harrison J. (1981). Membrane state and pollen viability. Ann. Bot. 47, 759-770.

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