In situ measurement of molecule mobility in mucilage polysaccharide gels from different species

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Introduction

On imbibition, the seeds of certain species form a polysaccharide hydrogel, termed mucilage. In *Arabidopsis thaliana* and *Camelina sativa*, this mucilage is composed of 2 layers, the outer being water-soluble while the inner is tightly attached to the seed surface. Determining the macromolecular properties of mucilage polymers in the inner mucilage layer is usually achieved by detaching the polysaccharides from the tegument either by physical or enzymatic methods, which alters or eliminates the structure. In order to characterize these properties, we have developed three non-destructive microscopy techniques. Based on fluorescence excitation or nuclear magnetic resonance, these in situ methods aim at investigating the physicochemical properties of mucilage polysaccharides that could affect accessibility and mobility of molecules within the hydrogel. This work presents results obtained on Arabidopsis and Camelina seeds comparing their inner mucilage properties.

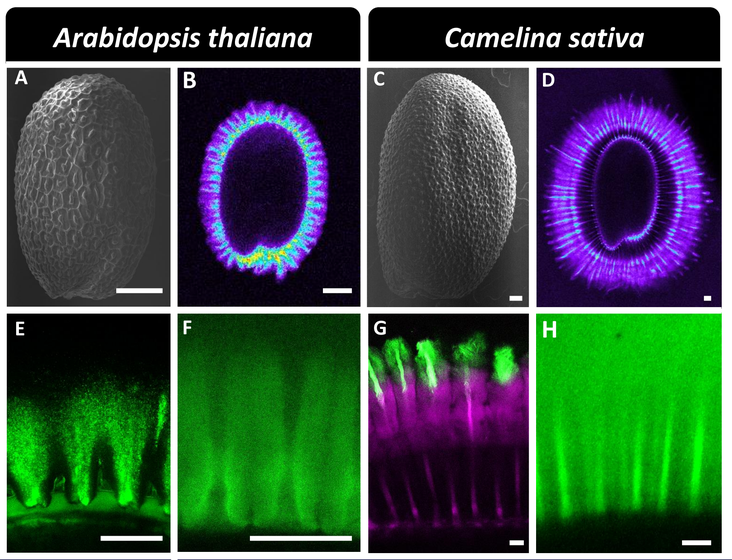

Figure 1. The organisation of mucilage constituents in Arabidopsis (A, B, E and F) and Camelina (C, D, G and H) seeds. (A) and (C) showing seed coat features, (B) and (D) after cellulose staining of inner mucilage with 0.01% pontamine fast scarlet 4b in 50mM NaCl. Immunolabelling indicates a difference in the heteromannan localisation (green in pictures (E) and (G), magenta showing cellulose staining).FITC polysucrose 40kDa shows differences in the permeability of the inner mucilage. Scale bar: 100µm (A to D), 50µm (E to H).

Fluorescence Recovery After Photobleaching

Material: Seeds imbibed in water and then stained with fluorescent probes (FITC-polysucrose) of different sizes (20kD, 40kDa and 400kDa).

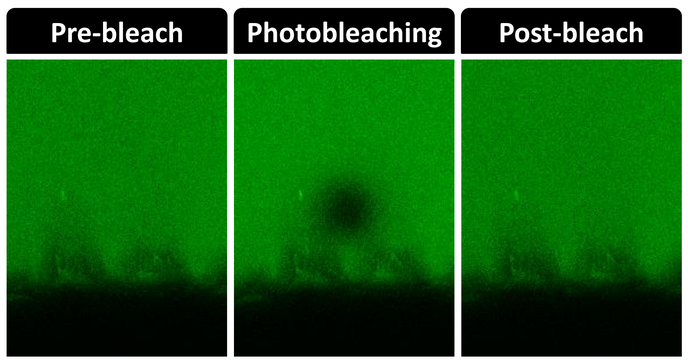

Figure 2. FRAP acquisition sequence. A Leica SP5 confocal microscope was used to achieve pre-bleach, bleach and post-bleach.

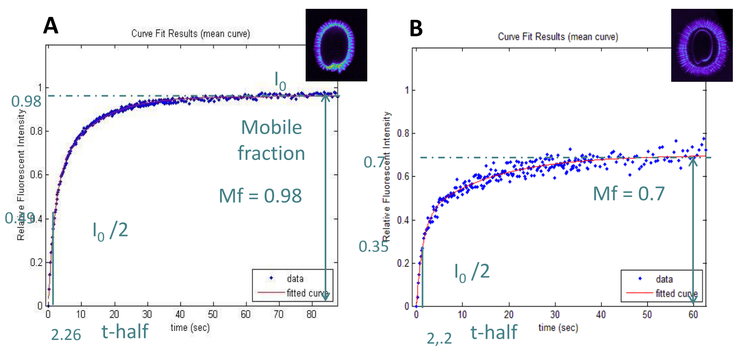

Figure 3. Camelina mucilage traps some molecules compared to this of Arabidopsis. Results of FRAP data analysis[3] after normalization and fitting the raw data using a biexponential algorithm shown in (A) for Arabidopsis and (B) for Camelina.

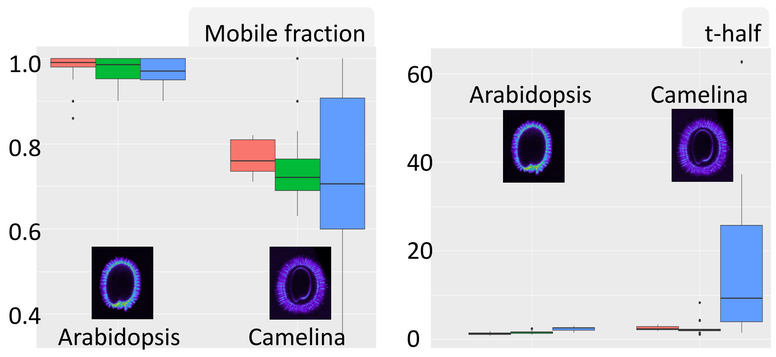

Figure 4. Differences in Arabidopsis and Camelina molecule mobility. **(**A) represents the mobile fraction of different sizes of fluorescent probe (pink: 20kDa, green: 40kDa and blue: 400kDa) in Arabidopsis or Camelina mucilage and (B) the t-half.

Conclusions: - FRAP can be to obtain information about molecule mobility; if molecule moves freely or is subject to steric hindrance.
- The physical properties of Camelina and Arabidopsis inner mucilage differ.

Fluorescence anisotropy

Material: Arabidopsis seeds imbibed in water and then stained with fluorescent probes (FITC-polysucrose) of different sizes (20kD, 40kDa and 400kDa).

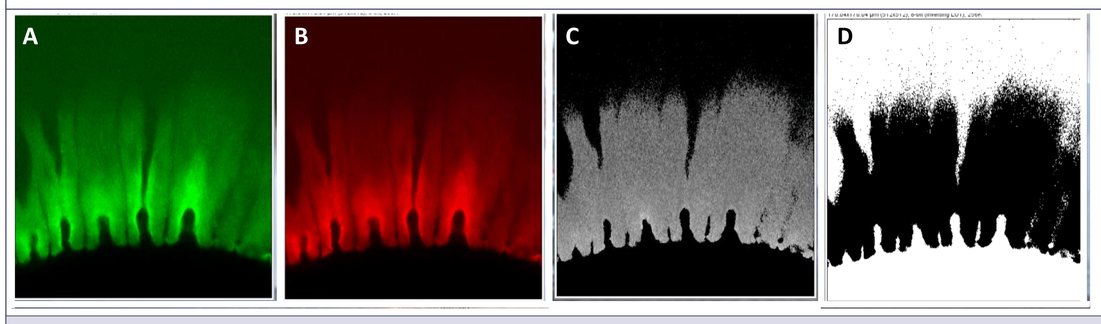

Figure 5. Anisotropy measurement[4]. Fluorescence emission intensity (A) perpendicular and (B) parallel to excitation plane, is analysed with imageJ to obtain (C) the anisotropy and (D) the maximum intensity threshold of the (C) image.

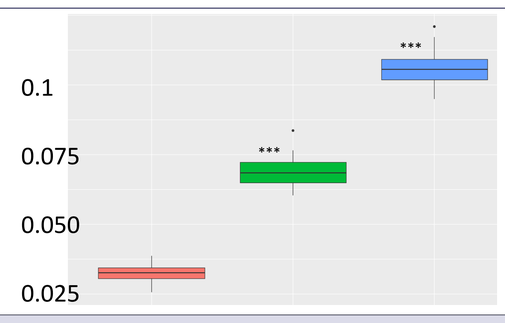

Figure 6. Anisotropy values obtained with different fluorophore sizes (pink: 20kDa, green: 40kDa and blue: 400kDa).

Conclusions: - Anisotropy measurement of molecule mobility provides information about hydrocolloid viscosity and molecular crowding
- Data acquisition is fast (< 5 min) allowing different samples to be compared in a single experiment
- This technique is independent of fluorophore concentration.

Magnetic resonance micro-imaging

Material: Camelina seeds imbibed in water without a probe molecule. Water mobility is measured through spatially localized T2 relaxation time.

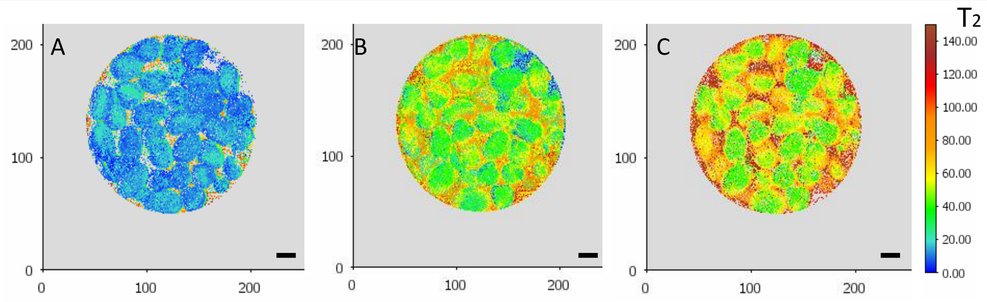

Figure 7. T2 maps of Camelina seeds imbibed in water. Data was acquired using the MSME sequence (Bruker, 11.7 T) with 128 echos, TE=2.96 ms, TR=1s. Resolution = 55µm²x1mm with a matrix of 256x256 voxels. (A) : distribution of the shorter T2 water component (0-20 ms) within seeds. (B), (C) : distribution of water and fat with T2 between 20-40 ms, surrouding ring with T2 around 60 ms is assigned to water in the inner mucilage layer. T2 of soluble mucilage in the water phase outside seeds exceed 120-140 ms. Scale bar: 1mm.

Conclusions: - MRµI allows quantification of water and fat in or outside seeds of diameter > 1mm².
- Rapid image acquisition (15 min) enable studies of the rate and evolution over time of water distribution and mobility in the inner mucilage layer.
- The first MRµ-images of Camelina indicate that measure of the water mobility and its change with time is possible with a resolution of 55 µm² radial slice.

Perspectives

Three complementary, non-destructive *in situ* methods have been developed which provide information about hydrocolloid organization and the rheological properties of seed mucilage. Each of them is rapid enough for the analysis of several different seeds in a single experiment.

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ANR-14-CE19-0001-01

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