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Using Atomic Force Microscopy to study thylakoid membranes

During my stay in Barcelona, at the Universitat Autònoma de Barcelona, I had the opportunity to study thylakoid membranes with Atomic Force Microscopy (AFM). This is a new excellent technique which allows to gain more knowledge about chloroplasts.
It's already known that plants which emits isoprene are better protected against hot temperature stresses compared to the non-emitting plants. Furthermore isoprene emitting plants are able to keep photosynthesis values higher during heat stress.
The idea was to investigate the structure of the thylakoid's membranes of isoprene emitting and non-emitting species with AFM.
To perform this experiment wild type tobacco plants, who naturally do not emit isoprene, and transgenic isoprene emitting tobacco plants were used.
De-enveloped chloroplasts from different plants were isolated. Once isolated, a layer of de-enveloped chloroplasts was distributed over a glass coverslip in order to be measured with Atomic Force Microscope.
The following measurements were performed, at different temperature points and, with and without bovine serum albumin (BSA):
-the Young's modulus, that is a measure of the stiffness, of the thylakoid's membrane. Young's modulus is the elastic modulus, is a number that measures an object or substance's resistance to being deformed elastically (i.e., non-permanently) when a force is applied to it;
- the visco-elasticity of the membranes.
The temperature was controlled thanks to a Peltier element which was located under the sample.
Moreover we also imaged the chloroplasts with atomic force microscopy, scanning electronic microscopy (SEM) and transmission electronic microscopy (TEM) , in order to:
- show that chloroplasts are de-enveloped;
-to measure the length of grana's stacks, which can vary between samples and maybe contribute to the measuring parameters;
-to highlight a different ultrastructure.
Preliminary results showed that the Young's modulus is different between isoprene emitters and non-emitters. I'm still analyzing all the data collected, but the preliminary results seem very promising.
Surely, these will allow us to better understand why isoprene emitting plants are better protected against heat stress.