Protocol for detecting the berry skin surface using a cryo-scanning electron microscope

Background

The berry skin texture and its wax coating have a considerable influence on the susceptibility of a grape variety tograpе variety to gray mold rot caused by *Botrytis cinerea*. In the INTERREG project WiVitis, this wax coating on berries of fungus-resistant grape varieties (PIWIs) and traditional grape varieties is being investigated using various methods. In addition to the use of sensor-based methods, a cryo-scanning electron microscope (cryo-SEM) is also used. This allows the berry skin surface to be magnified up to 40,000 times in order to make the tiny structures of the wax coating visible to the human eye and to examine them for structural differences.

Sample preparation

First, a 3-5 mm large, flat piece of the berry skin is cut out of the preselected berry with a scalpel and fixed on a sample plate together with other berry skin preparations.

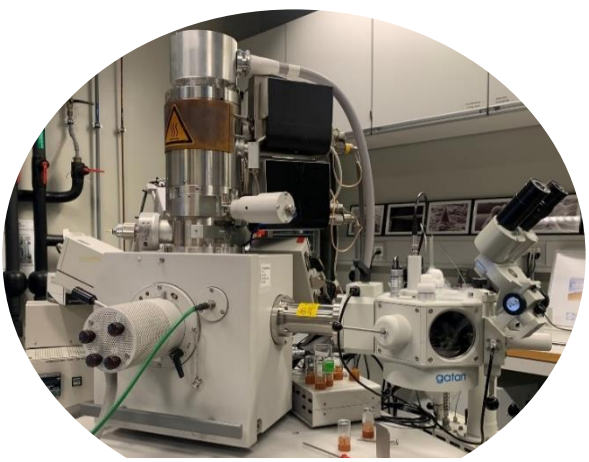
A very high vacuum at room temperature is present in the SEM. This would cause the water in the berry skin preparation to evaporate immediately and the waxes to collapse. The preparations must therefore be shock frozen in liquid nitrogen at -210°C in advance. At this optimum freezing rate, no ice crystals will form, which means that the cells and waxes of the samples stay intact and can be recorded true to the original.

Preparation of the berry skin sample under the reflected light microscope

Function in the cryo-SEM

Our sample plate with the ten frozen pieces of berry skin can now be placed in the cryo unit. As our project partner NI Lab Basel has a viewing window, it is possible to observe how the frozen samples are coated with approx. 30 nm of gold (other electrically conductive metals or carbons are also possible). This prevents the samples from becoming charged under the electron beam later in the SEM, stabilizes them and improves their imaging secondary electron signals.

Frozen samples in the cryo unit



Cryo-SEM of the Nano Imaging Lab Basel

The prepared samples are then transferred through the airlock into the SEM. At approx. -150°C, the electron beam is guided over the sample line by line and interacts with the sample surface at every point. The resulting signals are measured with detectors, which are used to visualize the composition of the sample surface. Depending on the number of electrons measured at a point, it will appear lighter or darker on the PC screen. The results are impressive, high-resolution photos of the skin surfaces of various grape varieties.

WiVitis

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