## Microscopy techniques for visualizing nanomaterial in plant tissues

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To date, the application of nanotechnologies in agriculture is increasing. Indeed, conventional pesticide formulations have major drawbacks in relation to efficacy, toxic effects and environmental impact of nano-formulated systems, such as nanopesticides or nanofertilizers, that could provide targeted/controlled release of agrochemicals and enable more complete biological efficacy without overdosing. The combination of novel antibacterial nanomaterials and the early identification of infectious microorganisms appear to be a promising strategy for the treatment of bacterial infections in plants. Indeed, the rapid spread of some epidemic like for example the infection of olive oil by Xylella fastidiosa, has caused serious damage in terms of production and economy.

Nowadays microscopy techniques have become an essential tool for the visualization of nanomaterials in plant tissues, for monitoring the efficacy and the safety of the nano-phytodrug. Through a detailed analysis by transmission and scanning electron microscopy (TEM, SEM) and X-ray nano-tomography of the different plant parts, i.e., leaves, stems and roots, the presence and the bioaccumulation sites of nano-phytodrugs can be identified and visualized.

The microscopic procedures for SEM-TEM (STEM, Zeiss Auriga) will be discussed for a better evaluation of the internalization and biodistribution of different nanoformulations, like CH-nanoFos, nanolignin, etc. The stages of sample preparation, such as chemical fixation, contrast, dehydration and embedding in epoxy resin blocks will be highlighted, strengthening the optimization to obtain satisfactory results in terms of image quality and resolution.

Finally an extensive discussion of the results obtained by using a latest generation nanotomograph Xradia Versa 610 (Carl Zeiss GmbH), which guarantees the non-destructiveness of the analyzes, the achievement of morphological and structural information in three-dimensional space at submicrometric resolution ensuring a study of the sample in its entirety.