

LOCALIZATION OF NANOPARTICLES IN PLANT TISSUES: POTENTIAL USE AS SMART DELIVERY SYSTEMS

A. Pérez-de-Luque^{1*}, R. Fernández-Pacheco², M.J. Coronado³, E. Corredor³, P.S. Testillano³, M.C. Risueño³, C. Marquina⁴, M.R. Ibarra^{2,4}, D. Rubiales⁵, P. González-Melendi⁶

¹IFAPA-CICE (Junta de Andalucía), CIFA "Alameda del Obispo", Área de Mejora y Biotecnología, Apdo. 3092, E-14080 Córdoba, Spain; ²Instituto de Nanociencia de Aragón, Universidad de Zaragoza, Edificio Interfacultativo II, Pedro Cerbuna 12, 50009-Zaragoza Spain; ³CSIC, Centro de Investigaciones Biológicas, Dep. Plant Development and Nuclear Organization, Ramiro de Maeztu 9, E-28040 Madrid, Spain; ⁴CSIC-Universidad de Zaragoza, Instituto de Ciencia de Materiales de Aragón-Departamento de Física de la Materia Condensada, Pedro Cerbuna 12, 50009-Zaragoza, Spain; ⁵CSIC, Instituto de Agricultura Sostenible, Alameda del Obispo s/n, Apdo. 4084, E-14080 Córdoba, Spain; ⁶Centro de Biotecnología y Genómica de Plantas. Universidad Politécnica de Madrid. E.T.S. Ingenieros Agrónomos. Ciudad Universitaria s/n. 28040-Madrid. Spain

* Corresponding author: bb2pelua@uco.es

In the last years, the nanotechnology is revolutionizing many areas of science and technology, and agriculture can not to be kept away from it. In this way, many applications have been suggested, including nanosensors and nanodevices, bioselective surfaces, nanomaterials, etc. [1, 2]. We have begun the development of a research line in order to find practical applications of nanotechnology in agriculture. Our goal is to find nanosystems which can be easily used as smart delivery systems in plants and select the most suitable ones according to the substances to be introduced into the plants (drugs, nucleic acids, etc.).

The great potential of nanoparticles as delivery systems to be directed to specific targets in living beings has been first explored for medical uses [3, 4]. The same principles can be applied to plants, for a broad range of uses in particular to tackle infections with nanosystems tagged to pesticides or other substances for efficient and local treatments, thus reducing the dose of chemicals released to the environment. For that reason, the first systems we have selected for preliminary assays are carbon coated magnetic nanoparticles [5, 6]. The magnetic core allows allocation of the nanoparticles in the site of interest (affected tissues) using small magnets. On the other side, the carbon encapsulation provides biocompatibility and a large adsorption surface. Thus, different types of molecules can be adsorbed on the carbon coating [5, 6]. It is also possible to functionalise with and/or conjugate the coating of the particles to different biomolecules, which is of great interest for its use as a smart delivery system to target specific tissues.

In order to explore the benefits of nanotechnology applications in agriculture, the first level of our research is to achieve the correct penetration and transport of the nanoparticles through the plant. In this context, the unambiguous localization of the particles in the plant tissues is pivotal. Our first work is aimed to put forward a number of tools for the detection and analysis of magnetic nanoparticles introduced into plants by using different techniques, ranging from conventional light microscopy to confocal and electron microscopy.

We have inoculated *in vitro* growing plants with a ferrofluid composed of carbon-coated magnetic nanoparticles. Tissue samples were then collected, fixed, cut and observed with different microscopical techniques to detect the presence of nanoparticles. These techniques include conventional light microscopy, fluorescence microscopy, confocal scanning laser microscopy and electronic microscopy, combined with different fixation and/or embedding processes.

References:

- [1] Scott N and Chen H, Cooperative State Research, Education and Extension Service, The United States Department of Agriculture, (2003).
- [2] Joseph T and Morrison M, www.nanoforum.org, (2005).
- [3] Alexiou C, Wolfgang A, Klein R J, Parak F G, Hulin P, Bergemann C, Erhardt W, Wagenpfeil S and Lübe A S, *Cancer Res*, **60** (2000) 6641-8.
- [4] Alexiou C, Jurgons R, Schmid R, Hilpert A, Bergemann C, Parak F and Iro H, *J Magn Magn Mater*, **293** (2005) 389-93.
- [5] De Teresa J M, Marquina C, Serrate D, Fernández-Pacheco R, Morellon L, Algarabel P A and Ibarra M R, *Int J Nanotechnology*, **2** (2005) 3-22.
- [6] Fernández-Pacheco R, Ibarra M R, Valdivia J G, Marquina C, Serrate D, Romero M S, Gutiérrez M and Arbiol J, *Technical Proceedings of the 2005 NSTI Nanotechnology Conference and Trade Show*, **1** (2005) 144-7.

Figures:

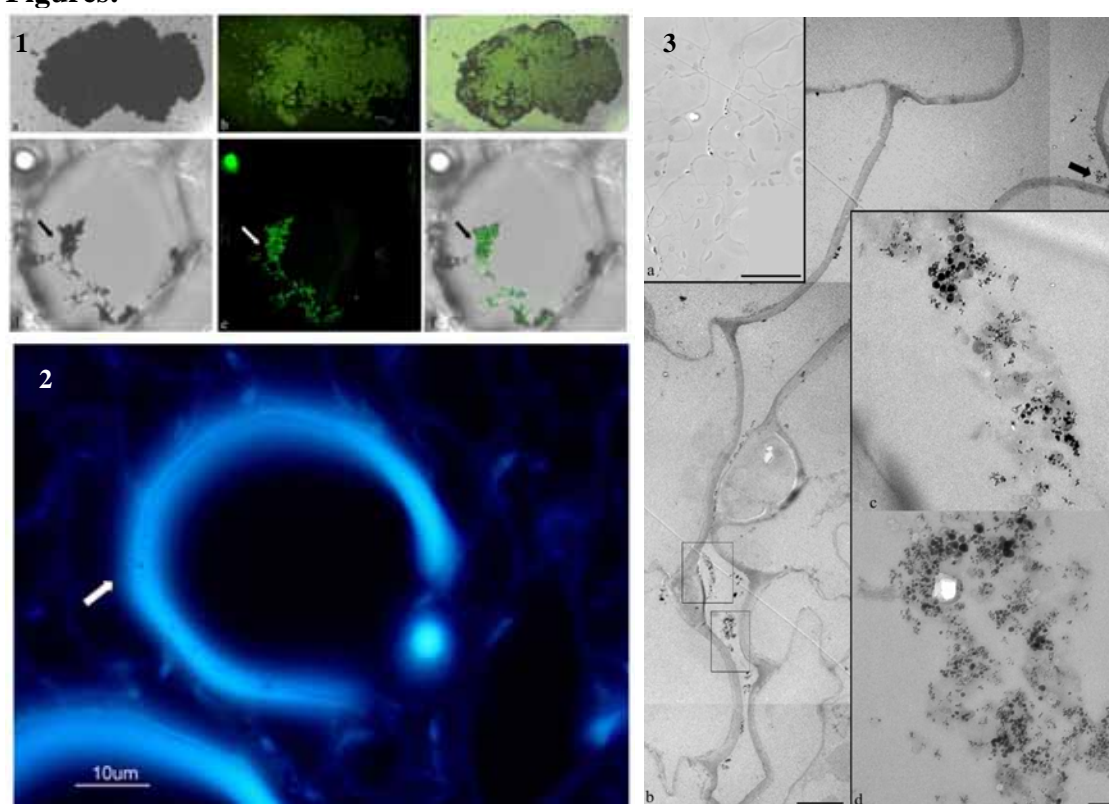


Figure 1: Detection of aggregates of nanoparticles in the solution injected into the plants on projections of 3D confocal stacks under Nomarski (a) and reflection (b). 1(c) shows the overlay between (a) and (b) with an almost complete colocalisation. Aggregates of nanoparticles were also detected into a cell of the stem (arrows), after 72 hours at the position of the magnet, on vibratome sections observed on the confocal microscope: (d) Nomarski, (e) reflection and (f) overlay.

Figure 2: Detection of nanoparticles on thick sections (7 µm) of paraffin-embedded plant tissues. The identified particles correspond to non-fluorescent areas (arrow) within the autofluorescent cell wall of xylem cells under an epi-fluorescence microscope.

Figure 3: Nanoparticles detected on correlated light and electron microscopy imaging of the same specimen after 24 hours at the injection site. a) the nanoparticles are seen as beads on a string along the cells on the outer side of the cell wall by phase contrast on a light microscope. b) the same area as in (a) imaged on the transmission electron microscope where electron dense areas can be seen (arrows). c and d) higher magnification of the boxed areas in (b) in which aggregates of nanoparticles can be clearly recognised in the apoplast.