Proceedings of the LXV SIGA Annual Congress
*Piacenza, 6/9 September, 2022*
ISBN: **978-88-944843-3-5**

**Poster Communication Abstract – 7.26**

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## EXPLORING NANOTECHNOLOGY-BASED STRATEGIES FOR GENETIC MODIFICATIONS IN HIGHER PLANTS

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*magnetic nanoparticles, pollen, Solanaceae, DNA and Protein Delivery, genome editing*

In the last decade, approaches for inducing genetic modifications in higher plants have been revolutionized by the advent of genome editing tools, especially those based on CRISPR/Cas technology, which allows precise and facile targeting of specific genes. Nevertheless, several bottlenecks reduce the efficiency and the scope of such tools, and their wide implementation in breeding. Among them, efficiency of conventional methods for transformation and regeneration is highly variable among crops and, even for responsive species, among varieties/lines. Hence, alternative methods for delivery of genome editing toolkit, not relying on tissue culture approaches, are highly desirable. The use of various types of nanoparticles can be explored to shuttle nucleic acids, proteins, or their complexes, into a range of tissues and cells to achieve desired genetic modifications without passing through a tissue culture phase.

We are using iron oxide magnetic nanoparticles (MNPs) with a mean diameter of 13 nm as carrier of DNA plasmids and other molecules in pollen grains. Tentatively, such relatively small particles can diffuse into target cells either spontaneously or forced by an external magnetic field. Treatments with different concentrations of MNPs did not affect pollen morphology and

germination, suggesting that, in the conditions used, MNPs can be employed as non-toxic carriers in plants. Interestingly, we observed that pollen grains of potato, tomato and an interspecific Solanum spp. somatic hybrid treated with TAMRA-conjugated MNPs showed a strong fluorescent signal, suggesting that nanoparticles at least deposited on exine. Assessment of either spontaneous or forced internalization as well as MNP subcellular localization are under way. Moreover, we are presently optimizing the conditions for loading a GFP-expressing plasmid on positively charged MNPs and for recovery of treated pollen for subsequent pollinations.