Organelle genomes exposed to different ENMs in *Arabidopsis thaliana*: structural maintenance, function and abundance

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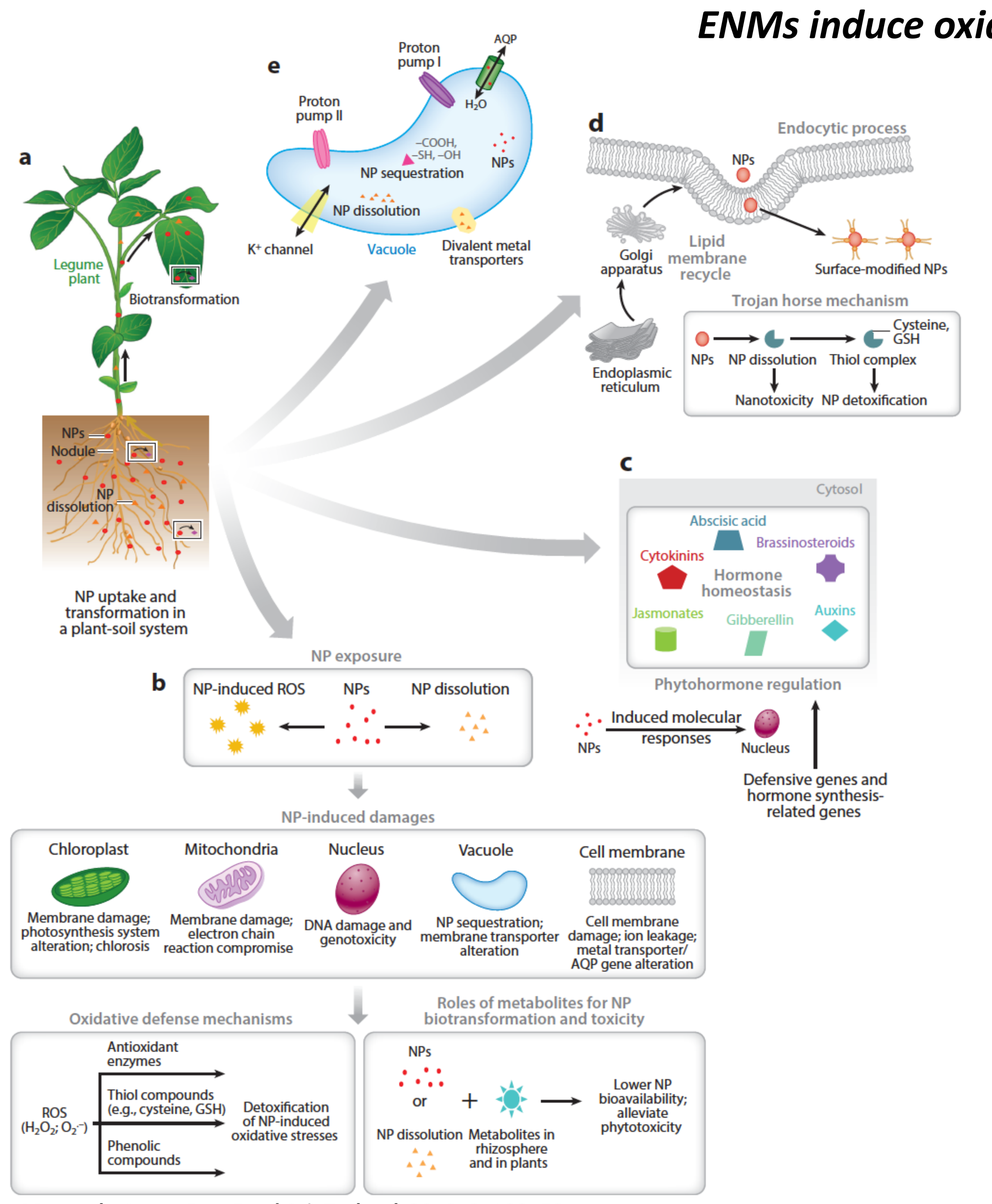
One of the challenges restricting the commercial development of engineered nanomaterials (ENMs) is the perceived lack of understanding of their negative environmental effects. Although evidence has been accumulating on the biological effects of ENMs at the level of cells, tissues, and organisms, wide differences in experimental design confound a systemic analysis. Importantly, mitochondria and chloroplast are not only the cellular energy sources but also have important regulatory and developmental roles in cell function. Experimental data has shown that mitochondria and chloroplasts are sensitive targets of after metal-based ENMs exposure. The aim of this work was to examine mechanistically and compare mitochondria and chloroplast involvement in response to a number of different ENMs at a range of concentrations, both in terms of their functionality and their organellar DNA replication, as measured by specific genes of interest. The correlation of physiological endpoints with organelle functionality and the organellar DNA copy number aims to provides novel insights into the organelles involvement in plant response to ENMs exposure. This work highlights that organellar genomes can be subjected to stoichiometric or sub-stoichiometric shifts as a morpho-functional response to the physiological imbalances caused from the ENM exposure.

ENMs induce oxidative stress response in plants



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Mitochondria and chloroplast in higher eukaryotes are not only the energy power plant of the cells but have an important regulatory and developmental role.

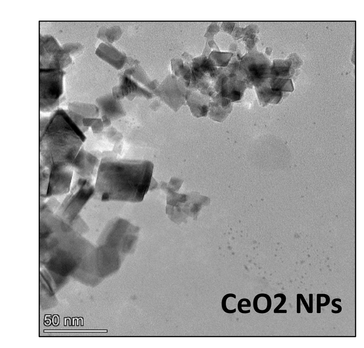
Experimental data have shown that mitochondria and chloroplasts are between the primary targets of metal-based ENMs.

Effects on the various cellular compartments are able to trigger a series of responses to different stimuli (e.g. oxidative stress, abiotic and biotic stress).

Aim of the project

In order to assess the potential copy number variations in response to ENMs exposure, analysis of the structural effects on chloroplast DNA and mitochondrial DNA have been performed by qPCR. Analyses have been compared with physiological measurement related to organelle functionality.

ENMs	size (nm)	(ζ) Z-potential (mV)	(dh) hydrodynamic range (nm)
CdS QDs	<5	+ 15.8	178.7
ZnS QDs	<5	+ 61.6	1190
Fe_2O_3 NPs	<15	+ 3.8	978
Fe_3O_4 NPs	<10	+ 44.2	271.6
CeO_2 NPs	<25	+ 42.5	243.9



Experiment 1

CdS QDs treatments:

Time of exposure: 0, 10, 20 days of transplant on MS medium + CdS QDs

Concentration: 0, 40, 80, 150, 250 mg/L of CdS QDs

Metal ion comparison: 5.5, 11.5 mg/L of $CdSO_4$

Experiment 2

CeO_2, Fe_2O_3, Fe_3O_4 NPs, ZnS QDs treatments:

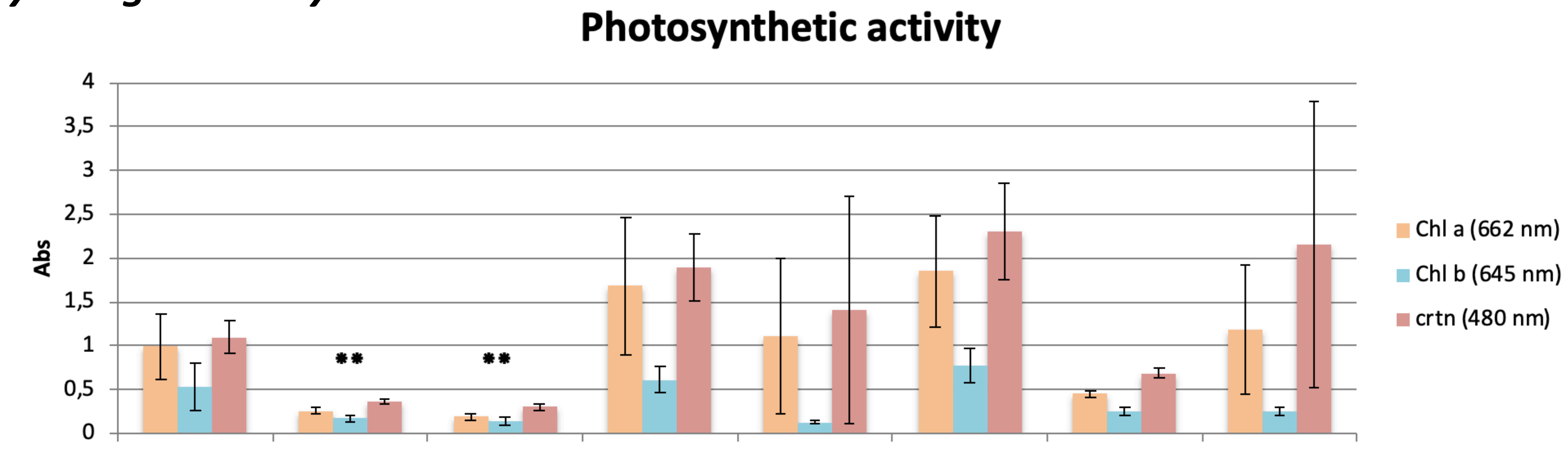
Time of exposure: 20 days of transplant on MS medium + ENMs or metal salts

ENMs	Conc. (mg/L)
CeO_2 NPs	500
Fe_2O_3 NPs	500
Fe_3O_4 NPs	500
ZnS QDs	500

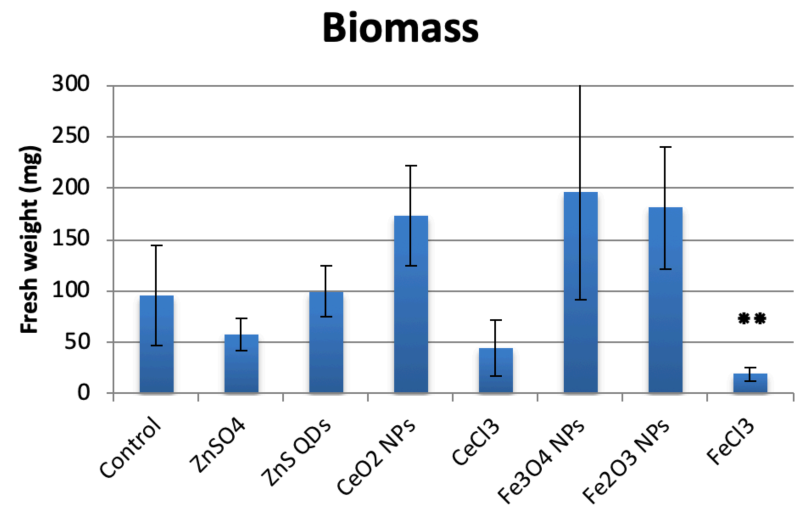
M salts	Conc. (mg/L)
$CeCl_3$	175
$FeCl_3$	75
$ZnSO_4$	175

Physiological analyses

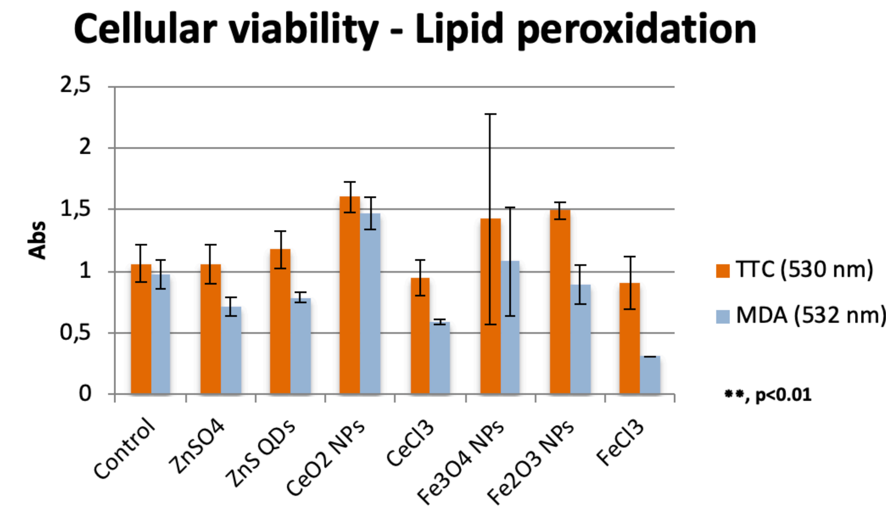
Photosynthetic activity



Biomass

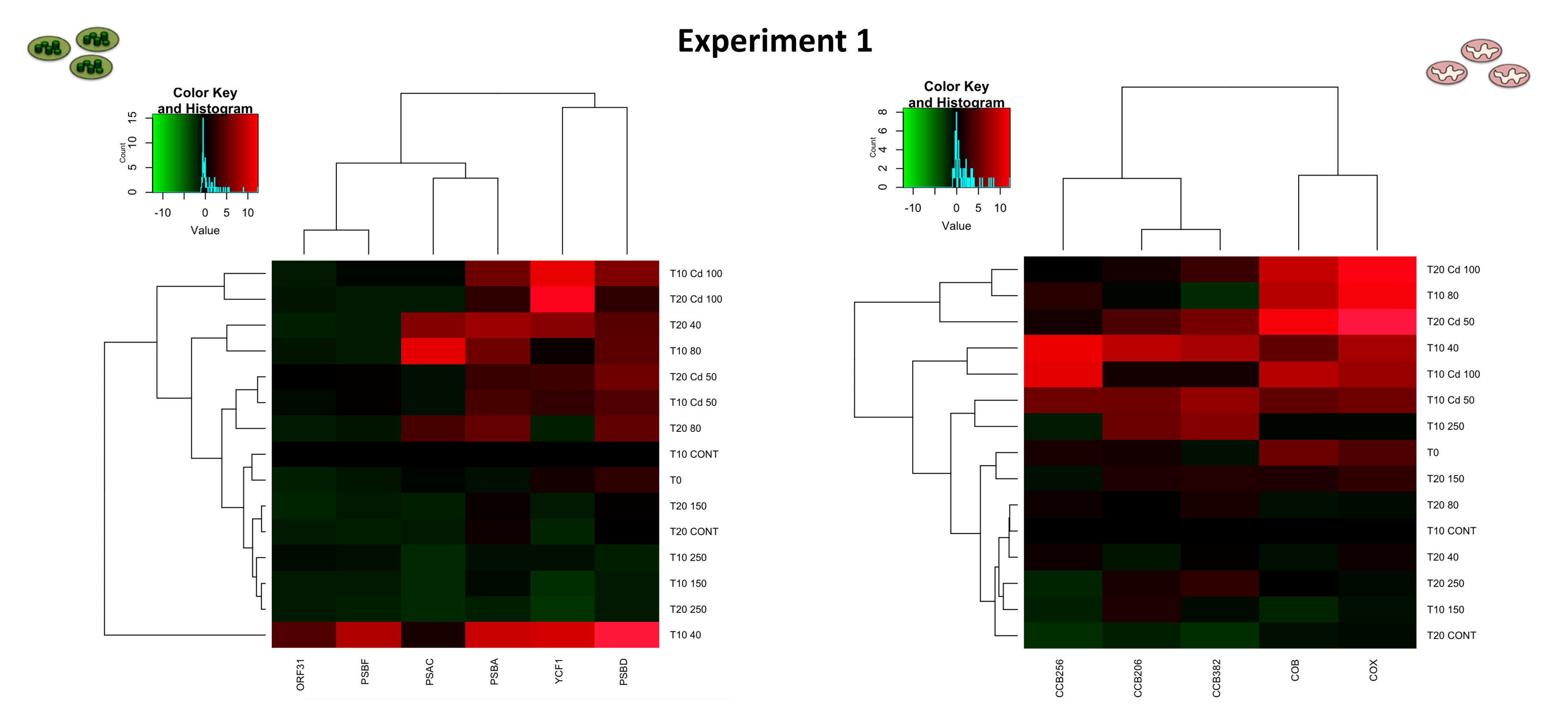


Cellular viability - Lipid peroxidation



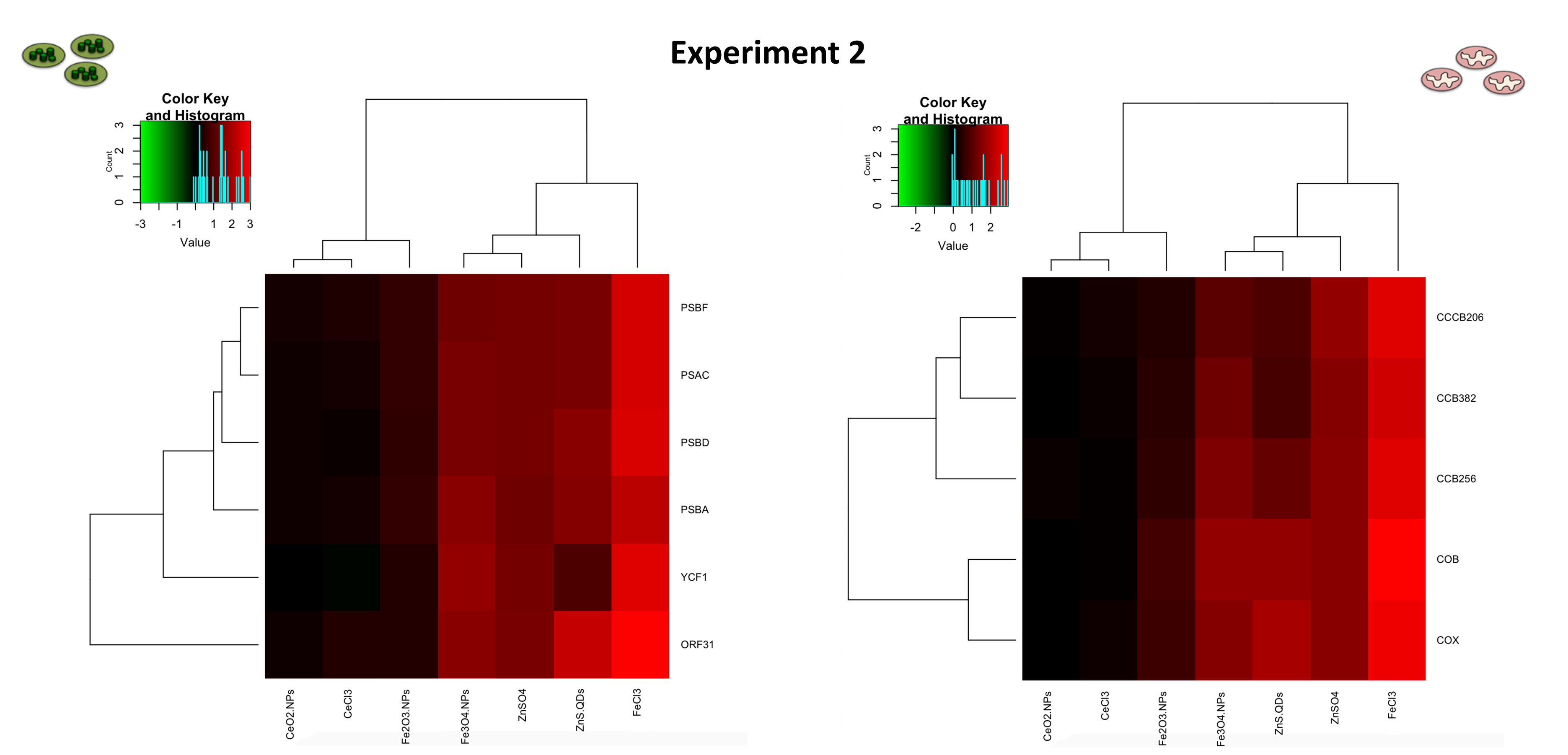
Real time qPCR analyses

Experiment 1



CdS QDs treatments showed a certain (geno)toxicity, that can be exemplified in a non-omogeneous amplification of the entire organellar genome, both in case of chloroplast and mitochondrial DNA.

Experiment 2



ENMs exposure mediate a chloroplast and mitochondrial DNA amplification, which result generally omogeneous. The differences observed may depends from the physico-chemical stability of the ENMs taken into account, but also from the potential biotransformation that can occur at the level of plant tissues.

Conclusions

The conditions of stress generated by the treatment may cause a potential variation in chloroplast and mitochondrial DNA copy number by mutation, replication or amplification processes.

Organellar DNA copy number variation can be utilized as potential biomarker in assessing and monitoring ENMs exposure in plants of agri-food interest.

Future perspectives

Effects of ENMs on gDNA at structural and functional levels, on different plant tissues, as well as the interplay between nucleus and organelles.

Chronic exposure, weathering and trans-generational effects (epigenetic inheritance) in more realistic scenarios of exposure.

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