DIFFERENTIAL PHYSIOLOGICAL RESPONSES OF *ARABIDOPSIS THALIANA* LINES TO AN EXCESS OF GREEN- SYNTHESIZED HEMATITE (α-Fe_2O_3) NANOPARTICLES

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Abstract

The increasing use of iron oxide nanoparticles (FeNPs) as nutritional supplements, pesticides and growth regulators in agriculture raises the concern about their widespread application potential ecological impact. However, there is a notable gap in the literature regarding their effects on plants with different epigenetic profiles. In this study, we compared the physiological and morphological responses of two *Arabidopsis thaliana* lines, wild type Col-0 and the methylation-defective mutant *ddm1-10*, to green-synthesized hematite (α-Fe_2O_3) nanoparticles to assess potential risks to plant systems. Our results showed that the addition of FeNPs to growth medium had no noticeable effect on seeding development or root length of Col-0 plants. In contrast, *ddm1-10* mutants exhibited reduced seedling growth and primary root length at all tested FeNP concentrations (up to 25 mg/L). Dark-grown seedlings of both *Arabidopsis* lines showed no observable changes in hypocotyl elongation upon FeNP exposure, suggesting the absence of hormone-dependent responses. Perls staining revealed iron accumulation at the root surface, indicating that excessive FeNPs likely deposit externally and may hinder iron uptake from the medium. This pilot study highlights the importance of epigenetic background in plant responses to nanomaterials and offers preliminary insights into the potential biological risks associated with agricultural use of green-synthesized FeNPs.

Material and methods

PLANT MATERIAL

Arabidopsis thaliana seedlings from the following lines were used:

- Wild type (Col-0) - baseline methylation status;
- Methylation-defective mutant (*ddm1-10*) – progressive, genome-wide reduction in methylated cytosine levels across generations (up to 70%).

ROOT GROWTH ASSAY

Seeds were surface sterilized, stratified for 2 days in the dark at 4°C, and germinated on half-strength Murashige and Skoog (½ MS) medium (pH 5.7) solidified with 1.0% agar. Experimental treatments included ½MS medium supplemented with 25 to 200mg/l green-synthesized hematite (α-Fe_2O_3) nanoparticles (Fe_2O_3-NPs). Plates were incubated in a growth chamber at 22°C and monitored for radicle emergence daily using an Epson Perfection V850 Pro scanner, and primary root length was measured using ImageJ software (version 1.54g).

HYPOCOTYL ASSAY

Hypocotyl length was determined after 120 hours of growth. Measurements were conducted using ImageJ (version 1.54g).

Fe HISTOCHEMICAL STAINING ASSAY

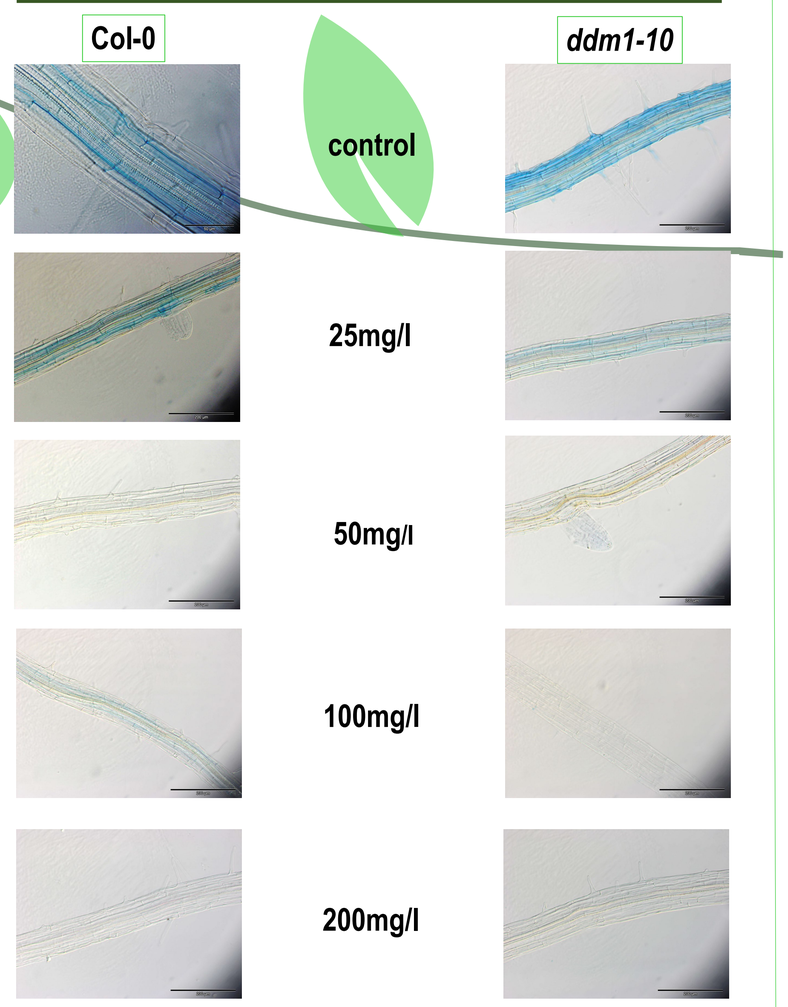
Perls staining (without DAB intensification) was used to detect labile (non-heme) Fe^{3+} in roots following the protocol of Brumbarova and Ivanov (2014). Seven-day-old seedlings were fixed in methanol:chloroform:glacial acid solution then infiltrated in 4% HCl and 4% K-ferrocyanide (Perls reagent) for 15 min. Samples were examined under an Olympus BX51 upright microscope, equipped with differential interference contrast (DIC) optics and XC50 digital microscope camera.

CONCLUSIONS

1. **DNA hypermethylation is involved in plant growth inhibition under stress conditions caused by excess Fe_2O_3-NPs.**
2. **An excess of Fe_2O_3-NPs in the medium likely leads to their accumulation on the root surface, limiting Fe^{3+} uptake and reducing nutrient absorption from the growth medium.**
3. **The excess of Fe_2O_3-NPs does not promote hypocotyl elongation and did not induce the formation of exaggerated apical hooks in plants.**

Results

Fe^{3+} detection in the *Arabidopsis* roots using the Perls stain

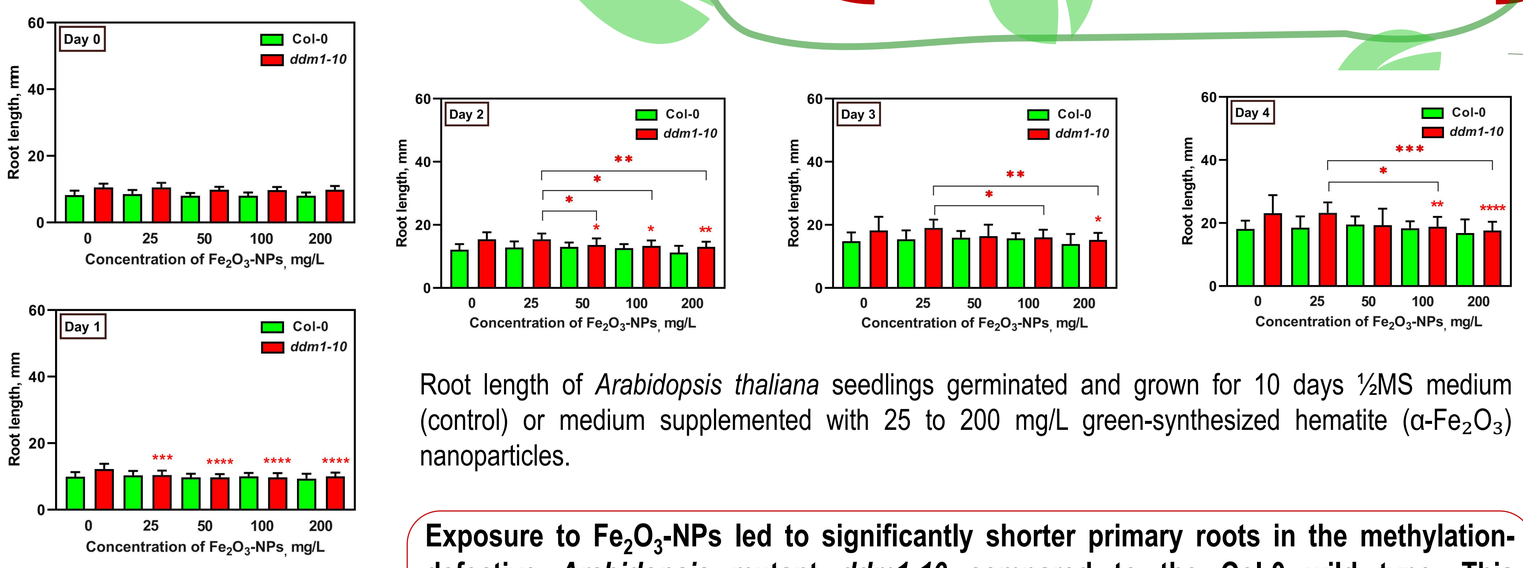


Suppression of Fe^{3+} accumulation in Fe_2O_3-NPs treated roots of 7-day-old seedlings of Col-0 and *ddm1-10* mutant as indicated by Perls stainng.

In the roots of Arabidopsis lines Col-0 and *ddm1-10*, Perls staining revealed Fe^{3+} accumulation in the endodermis and vasculature under control conditions and at low Fe_2O_3-NP concentrations (25 mg/L). However, at higher Fe_2O_3-NP concentrations, only weak Fe^{3+} signal was observed in the root meristem of both genotypes. These results suggest that excess Fe_2O_3-NPs may accumulate on the root surface, potentially obstructing iron and nutrient uptake from the growth medium.

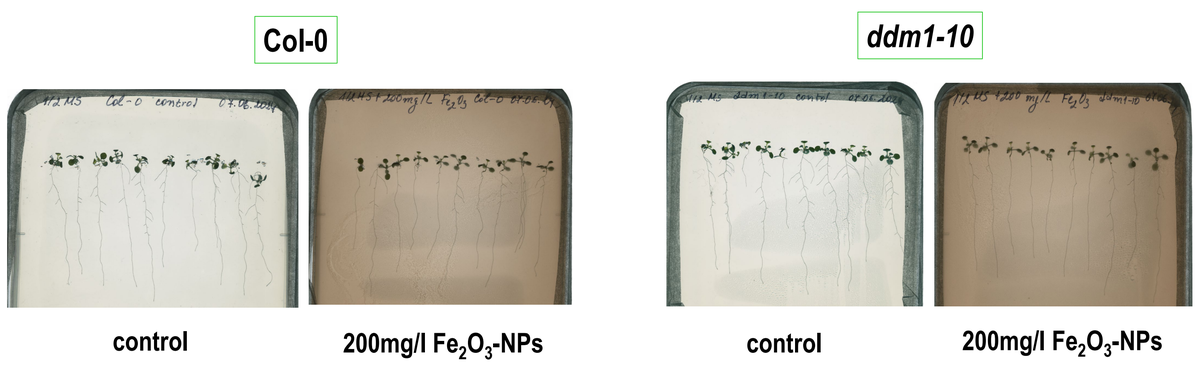
Results

Fe_2O_3-NPs-induced stress triggers morphological responses in the roots of the methylation-defective mutant *ddm1-10*



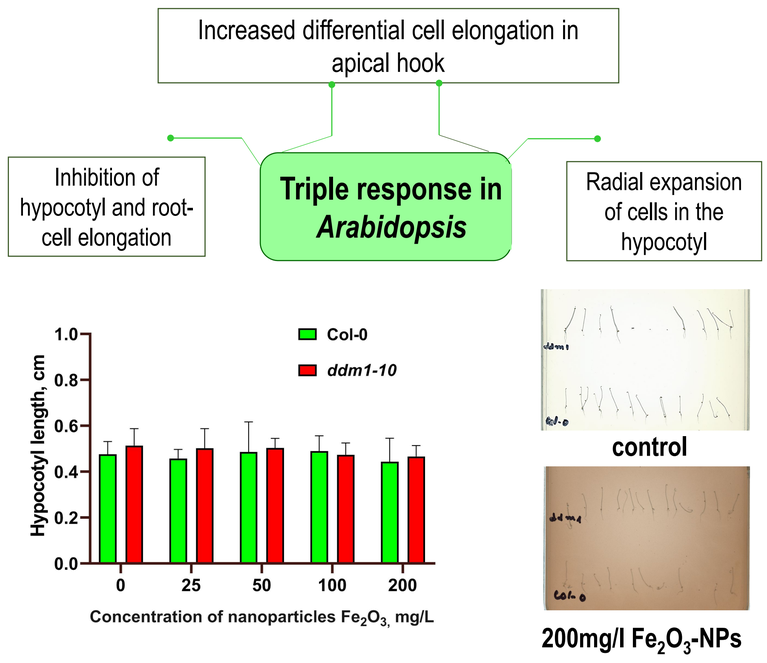
Root length of *Arabidopsis thaliana* seedlings germinated and grown for 10 days ½MS medium (control) or medium supplemented with 25 to 200 mg/L green-synthesized hematite (α-Fe_2O_3) nanoparticles.

Exposure to Fe_2O_3-NPs led to significantly shorter primary roots in the methylation-defective *Arabidopsis* mutant *ddm1-10* compared to the Col-0 wild type. This observation indicated that DNA methylation status contributes to the differences in plant stress response between normal and hypermethylated *Arabidopsis* genotypes under excess Fe_2O_3-NPs conditions.



Root growth phenotype of the wild-type (Col-0) and the methylation-defective *Arabidopsis* mutant *ddm1-10* under excess Fe_2O_3-NPs conditions 10 days after germination.

Assessment of triple response induction by green-synthesized Fe_2O_3-NPs in *Arabidopsis* seedlings for exploring hormone-dependent effects



Hypocotyl length of etiolated wild-type Col-0 and *ddm1-10* seedlings grown on ½MS medium with or without green-synthesized hematite (α-Fe_2O_3) nanoparticles (Fe_2O_3-NPs).

(A) Average hypocotyl lengths of Col-0 and *ddm1-10* seedlings exposed to increasing concentrations of Fe_2O_3-NPs.

(B) Representative images of 5-day-old etiolated Col-0 and *ddm1-10* seedlings grown in the dark under control conditions and with 200 mg/l Fe_2O_3-NPs. Sample size: n = 10 per line. Error bars represent standard deviation.

These results indicate that seedlings of both *Arabidopsis* lines did not show significant differences in hypocotyl length and did not develop hook-like structures at the apical region when grown in darkness. The presence of excess iron in the form of Fe_2O_3-NPs did not trigger a hormone-dependent response in *Arabidopsis* plants, regardless of their methylation status.