

## **Iron (Fe) and manganese (Mn) subcellular localization determined by TEM-EDS in embryo *Arabidopsis thaliana* mutants**

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In *Arabidopsis thaliana* seeds, the AtVIT1 transporter is involved in Fe influx into perivascular vacuoles whereas the AtNRAMP3 and AtNRAMP4 function in Fe retrieval during germination. The *vit1-1* and *nramp3nramp4vit1-1* mutants display an altered Fe pattern: Fe is accumulated in the vacuoles of abaxial cells of the cotyledons and radicle peripheral cells as revealed by Perls/DAB staining.

This raises the question of the compartment in which iron is stored in the absence of VIT1. To address this question, we examined the subcellular localization of Fe and Mn in cotyledons from wild-type, *vit1-1* and *nramp3nramp4vit1-1* triple mutant dried seeds using Energy Dispersive Spectroscopy (EDS) combined with Transmission Electron Microscope (TEM). This technique collects the X-rays spectrum emitted by plant sample sections bombarded with a focused beam of electrons to obtain a localized chemical analysis.

In *vit1-1* mutant embryos, Fe and Mn were both concentrated in subepidermal cell globoids, whereas Fe is localized in perivascular globoids in wild-type. In the *nramp3nramp4vit1-1* triple mutant Fe was detected at low level in globoids from subepidermal and perivascular cells although *vit1-1* and *nramp3nramp4vit1-1* have the same pattern revealed by Perl's/DAB staining.

TEM-EDS data complete the characterization of these mutants and open new questions of the iron localization and chemistry in the triple mutant. A cartography using STEM-EDX could solve this interrogation.