Investigating the Phytotoxicity of ZnO Nanoparticles and the Mechanism of Transport in the Prothallial Cells of Ceratopteris richardii

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In several instances, industrialization has led to increased contamination of agricultural fields with cytotoxic level of heavy metals. In seeded plants, the ZIP (ZRT- IRT-like Protein) family of transporters plays an integral role in the uptake, translocation, subcellular distribution and storage of selective metal cations. In cryptogams, the ZIP gene family remains uncharacterized. In this study, a full length orthologue of a ZIP gene (CrZIP1) was identified and isolated in the fern Ceratopteris richardii by screening the EST cDNA library and utilizing 3' RACE PCR. In silico analyses revealed that the gene codes for a putative membrane bound protein with eight transmembrane domains and numerous post-translation modifications. Phylogenetic studies showed that CrZIP1 as well as a small multigene family of putative ZIP genes from Physcomitrella patens and two paralogues from Selaginella moellendorffii are more closely related to ZIP4/IRT3 genes in Arabidopsis thaliana. Analysis of the EST cDNA libraries of Physcomitrella and Selaginella suggests differential regulation of the various paralogues of the ZIP genes identified in these organisms. RT-PCR analysis showed that the CrZIP1 gene is expressed at all stages of development and regulated transcriptionally by the level of zinc. Knockdown of CrZIP1 by RNAi resulted in gametophytes with reduced cell division and chlorophyll production in the prothalli when grown on C-Fern Media containing ZnO nanoparticles. Cell elongation and rhizoid formation were however promoted under these conditions. The results suggest that CrZIP1 may be the primary zinc transporter into prothallial cells and an initial knockdown of CrZIP1 may lead to an alternative transport of cytotoxic level of Zn into the cell.

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