Abstract

In the context of increasing environmental contamination with heavy metals, particularly cadmium (Cd), which has no biological function in living cells other than toxicity, this study aimed to evaluate the effects of 175 µM $CdCl_2$ after 24 h of exposure on the functioning of root meristems of *Vicia faba* L. var. *minor*, as well as to determine the extent to which thyme essential oil (TO; 0.03%) can protect cells against Cd toxicity. The initial response of the meristems was a substantial accumulation of Cd in the cells, accompanied by an overproduction of reactive oxygen species (ROS). Despite the activation of antioxidant mechanisms, including increased enzymatic activity and elevated levels of non-enzymatic antioxidants, as well as detoxification pathways based on thiol metabolism, this response proved insufficient, leading to enhanced lipid peroxidation and DNA damage. These disturbances were associated with disorganization of the ultrastructure of cellular organelles. Consequently, cells likely experienced impaired barrier function of the cell wall, related to difficulties in its reinforcement by fibrillar precursors transported in Golgi-derived vesicles, which were devoid of these components, as well as a probable deficiency of essential proteins, reflected by a reduced ribosome density in the cytoplasm. At the level of the genetic apparatus, disturbances in DNA replication and a decreased number of cells actively synthesizing DNA were observed. These changes were accompanied by activation of the MAPK signaling pathway and epigenetic alterations, including increased global DNA methylation and reprogramming of the histone code, leading to chromatin condensation and reduced global transcriptional activity. Together with decreased levels of key cell cycle regulators (CDKA and CycB), these disturbances contributed to mitotic arrest and a marked reduction in the mitotic index. In dividing cells, abnormal phosphorylation of histone H3 and cytoskeletal alterations, manifested as bundling and impaired rearrangement of microtubules and actin filaments, resulted in disorganization of the mitotic apparatus and inhibition of mitotic progression. Consequently, proliferation of meristematic cells was suppressed, leading to reduced root growth. Against this background, the protective effect of thyme essential oil (TO) was particularly evident, as TO alone did not destabilize the structure or function of meristematic cells. When applied during Cd exposure, TO not only partially reduced Cd accumulation in tissues but, more importantly, effectively mitigated its detrimental effects at multiple levels of cellular organization. The reduction of ROS levels in the presence of TO, along with a strong enhancement of both enzymatic and non-enzymatic antioxidant systems, led to a significant decrease in lipid peroxidation and double-strand DNA breaks. These coordinated effects resulted in a marked reduction of ultrastructural alterations in organelles, stimulation of cell wall precursor production in Golgi secretory vesicles, and enhanced transport of ribosomal subunits to the cytoplasm, associated with the formation of nucleolar vacuoles, thereby promoting translational activity, as evidenced by abundant ribosomes associated with the endoplasmic reticulum. At the level of nuclear regulation, TO attenuated activation of the MAPK signaling cascade and prevented epigenetic alterations, maintaining DNA methylation and histone modification patterns close to control levels, which supported the preservation of transcriptional activity. At the same time, TO stabilized both the replication and mitotic machinery, enabling sustained cell proliferation and proper root growth. In this context, the development and adaptation of the iPOND method for plant material provides new opportunities for the direct analysis of proteins associated with newly synthesized DNA and for further verification of the protective mechanisms of TO at replication forks. In summary, TO exerts a multifaceted protective effect, stabilizing cellular structure and essential processes in meristematic cells and effectively interrupting the cascade of Cd-induced toxicity, thereby confirming its potential as a natural agent supporting plant tolerance to cadmium stress.

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