Plants and their Pathogens

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Structural and immunoelectron microscopic studies on the effect of an electrostatic field in Triticum and Arabidopsis leaves

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The influence of electric fields (e-fields) including low-frequency pulsed e-fields and high-voltage electrostatic fields has been described for different organisms. Pulsed e-field treatment is frequently applied for the preservation of food, extraction of biological materials and reduction of bacterial contamination in wastewater [1]. Due to the broad range of applications (static, pulsed, high-, low intensities of e-fields) a wide spectrum of biological effects, e.g. inhibited/enhanced seedling growth, enhanced stress resistance, increased enzyme activities, increase in anthocyanin content, stimulation of the mitotic index, is documented for plants [2,3,4,5]. These differences in the applied kind of e-fields make comparisons of effects induced by e-fields difficult or even impossible. Moreover, data on influences of e-fields on the cell structural level are still rare or missing. In this study germinating seeds of *Arabidopsis thaliana* Col-0 and *Triticum sativum* Lam. were exposed to an electrostatic field with 2kV/cm intensity for five days. The effects of the applied e-field were evaluated immediately (intracellular localization of glutathione) and three weeks after the exposure (leaf area, cell size, cell ultrastructure) of plants to these conditions in climate controlled chambers.

The e-field exposure of seeds for five days during germination showed significant effects on the investigated plants. In *Triticum* the leaf area was significantly decreased (nearly 40%), whereas in *Arabidopsis* the leaf area did not change after e-field treatment when compared to untreated plants. Interestingly, the cell size was significantly decreased (up to 50%) in both *Triticum* and *Arabidopsis* leaves after e-field exposure. In addition light- and electron microscopical investigations of fixed and resin embedded leaves showed granular deposits in vacuoles of epidermal and mesophyll cells of e-field exposed *Triticum* plants (Figure 1). Besides these changes in vacuoles ultrastructural examination of both plants did not reveal further differences in subcellular structures and organelles between e-field treated and control samples (Figure 1c,d). Immunoelectron microscopic localization and quantification of glutathione in *Triticum* leaves five days after e-field exposure revealed significant differences in the gold labelling density of certain cell organelles. Plastids and nuclei were the most affected organelles showing significant reductions of the glutathione content in e-field treated samples (Figure 1e). The decrease of glutathione could be an indicator of oxidative stress on cellular level under these conditions.

This study clearly documents a statistically significant influence of a five day exposure of germinating seeds to a 2kV/cm electrostatic field on both cell physiology and cell structure. The effect is partly different in the investigated plants thereby indicating varying adaptations or responses to e-field conditions and stress, respectively.

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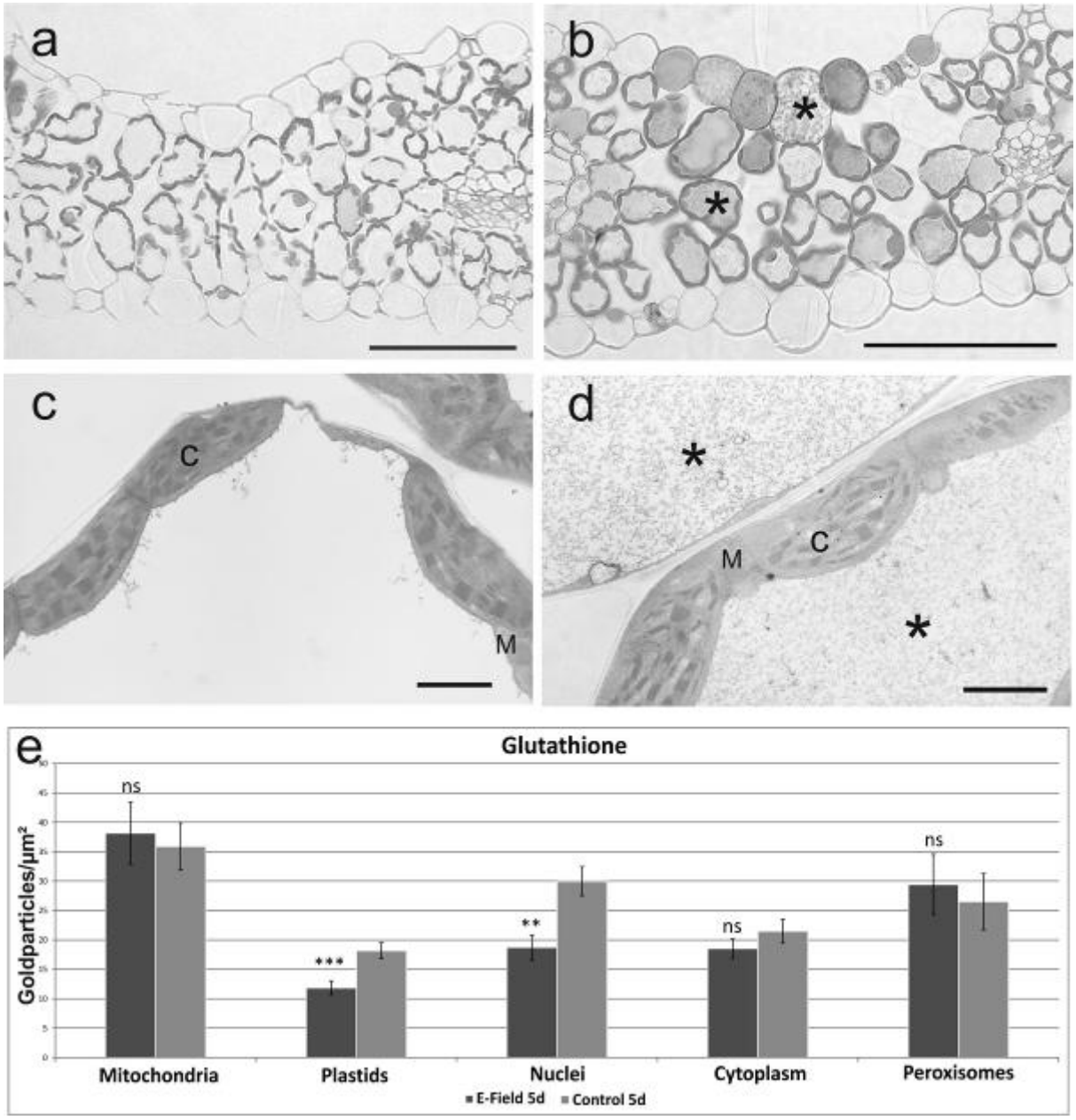

Figure 1. a,b: Semi-thin transverse sections of parts of three weeks old *Triticum sativum* leaves showing control samples (a) and granular deposits (*) in the vacuoles of epidermal and mesophyll cells of e-field exposed samples (b). Bars=100µm. **c,d**: Transmission electron micrographs of parts of mesophyll cells of three weeks old *Triticum* leaves showing inconspicuous chloroplasts (C) and mitochondria (M) in control (c) and e-field treated (d) samples. Dense granular deposits (*) are visible only in the vacuole of e-field exposed cells (d). Bars=2µm. **e**: Data represent mean values with standard errors of glutathione gold particle density per μm^2 in different cell compartments of mesophyll cells of five days old *Triticum* leaves. Significant differences were calculated with the Mann-Whitney U-test between control and e-field exposed samples. ns=not significant, (**) if $p<0.01$, and (***) if $p<0.001$. n >15 for peroxisomes and nuclei, n >50 for other cell structures.