Anatomical changes in Capsicum annuum leaves due to potentially toxic metals

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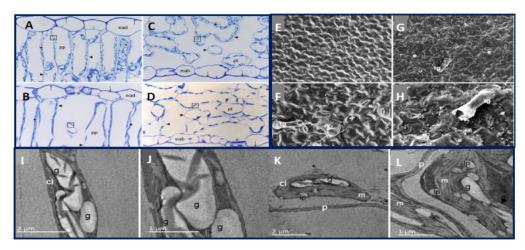
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**Background/objective:** The chili pepper accession UENF 1381, *Capsicum annuum*, belonging to the UENF germplasm bank is used as a model plant, because it has resistance to various diseases caused by *Xanthomonas euvesicatoria*, *X. gardneri* and *Colletrotrichum gloeosporioides*. In previous studies differences in leaf mineral composition of macro and micronutrients were detected in plants treated or not with potentially toxic metals. In order to understand the mechanisms of resistance and their interaction with these metals, we studied the anatomical structures of this accession, treated with Hoagland nutrient solution with and without some potentially toxic metals: Cd, Co, Cr, Li and Pb.

**Material/Methods:** Accession UENF 1381 was grown in pots containing sand and vermiculite mix, in a greenhouse at Campos dos Goytacazes, RJ, Brazil. The experimental design was a randomized complete block with two treatments and five replications, as follows: a) UENF 1381 + Hoagland, and b) UENF 1381 + Hoagland with addition of metals (Cd, Co, Cr VI, Li and Pb) [1]. Irrigation was performed daily. The leaves were collected in the second caulinar node from the apex with the aid of a 1cm diameter circular cutter. Samples were fixed, washed, dehydrated and further processed for Light Microscopy (LM), Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) [2].

**Results:** From LM analysis, it was observed that treatment without addition of metals caused an increase in cuticle thickness, cytoplasmic retraction (especially on adaxial side) and starch granules accumulation in both leaf faces (Fig. 1, A, B, C and D). Leaves treated with nutrient solution added with metals, when observed in SEM, showed a higher frequency of stomata and granular trichomes on the adaxial surface visibly with wider ornamentation (Fig. 1, E and G). Furthermore, in the same treatment, on the abaxial surface, there was an increase of the wax layer and with wider ornamentation in relation of treatment without metals (Fig. 1, F and H). The amounts of stomata and the arrangement of granular trichomes are similar in both treatments. From TEM analysis, it was possible to confirm the significant differences in the chloroplast ultrastructure. Starch granules were observed, as well as cytoplasmic retraction, thylakoid membranes and lipid droplets. The addition of metals resulted in lower accumulation of starch in the chloroplasts (Fig. 1, I, J, K and L). It also allowed a better observation of thylakoid membranes and oil droplets on the treatment with addition of metals (Fig. 1, K and L).

**Conclusion:** The treatment with addition of metals promoted anatomical and ultrastructural changes in UENF 1381 leaves. It reduced energy sources (smaller chloroplast with less starch) and weakened the physical barriers, since it reduced the cuticle barrier. The addition of metals possibly favors mechanisms that decrease the resistance of the studied genotype. Due to the structural differences observed, they can significantly influence defense mechanisms. Studies relating defense with structure are needed to better elucidate how the nutrient solution influences the defense mechanism.



**Figure 1.** Leaf surface of *Capsicum annuum* – UENF 1381. Treatments with Hoagland (A, C, E, I and J). Treatments with Hoagland and metals (B, D, G, H, K and L). Light micrographs of adaxial (A and B) and abaxial (C and D), bars: 10  $\mu$ m. Scanning electron micrographs of adaxial (E and G) and abaxial (F and H), bars: EG = 50  $\mu$ m, FH = 20  $\mu$ m. Transmission electron micrographs are I, J, K and L. • – indicative of primary starch; **A** - cytoplasmic membrane; **V** - cuticle; cl – chloroplast; e – stomata; eab – abaxial epidermis; ead – adaxial epidermis; g – starch granule; Ip – lipid droplets; m – thylakoid membranes; p – cell wall; pl – lacunar parenchyma; pp – palisade parenchyma; and tg – glandular trichoma.

## References:

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