The effects of copper and arsenic on the anatomy of wheat roots (*Triticum aestivum*, L.)

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Introduction

Roots are in direct contact with heavy metals in soils and therefore it is important to know in which ways roots are affected and how root anatomy changes due to heavy metal stress. Any reduction of roots affects the ability of water and nutrient uptake and deteriorates the physiological status of the whole plant. Young roots can be differentiated into a calyptra, a meristematic region, an elongation zone and a differentiation zone with root hairs. When cell differentiation is finished, we can distinguish a stele with vascular strands, a cortex and a root epidermis. Numerous studies investigate the negative effects of heavy metals on plants, but still researchers do not know the entire actions of heavy metals on organisms.

Our hypothesis is that heavy metals do not only harm plant metabolism but also adsorb to the cell wall and change wall properties and cell shape.Therefore, we want to answer the following questions:

Does arsenic affect root anatomy in any way?

Does copper influence pectins of the cell wall and therefore cell shape?

Are the anatomical changes specific to a certain metal (copper or arsenic)?

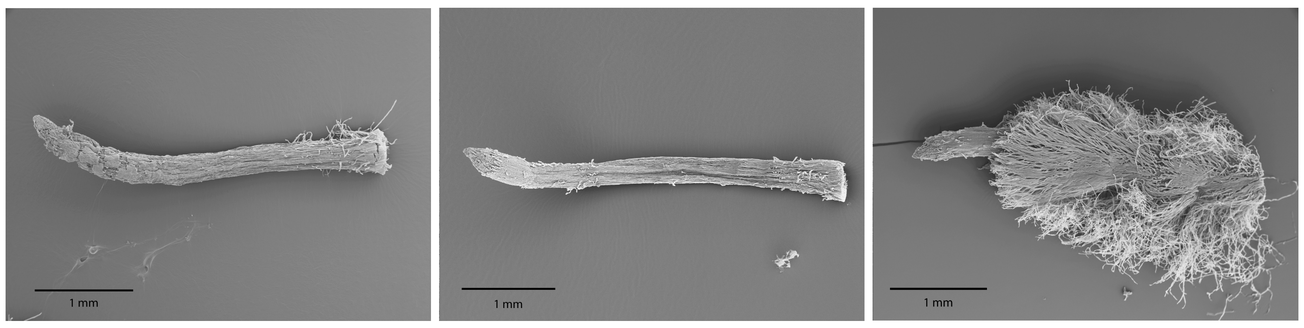

Fig.1: Roots treated with copper (left) and arsenic (middle) have hardly any root hairs in comparison to the control (right).

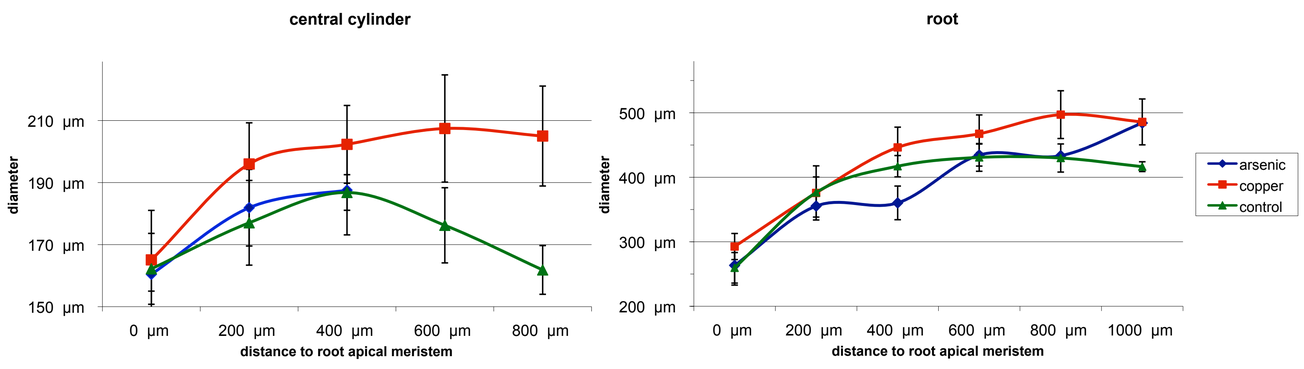

Fig.3: Comparison of the primary thickening of the roots of arsenic and copper treated plants with the control.

Material and Methods

We used *Triticum aestivum* (Poaceae) as model plant. Seedlings were cultivated in Knop solution supplemented with 10^{-3} M $NaHAsO_4$ or $3{,}2 \cdot 10^{-5}$ M $CuSO_4$, resulting in a 50% growth reduction. Roots were analysed after 2 days of growth. We determined the number of cells between 0 and 800 µm and 800 to 1600 µm distance to the root apical meristem and measured cell length at 800 µm and 1600 µm. Furthermore, we studied root morphology and element distribution using an EDX scanning electron microscope (Philips XL 20). In order to analyse anatomical changes, roots were embedded in paraffin and cross sectioned. Sections were stained with safranin and basic blue 140. We analysed the diameter of the whole root, the diameter of the stele, the development of xylem elements, the Casparian bandand the detachment of the root epidermis.

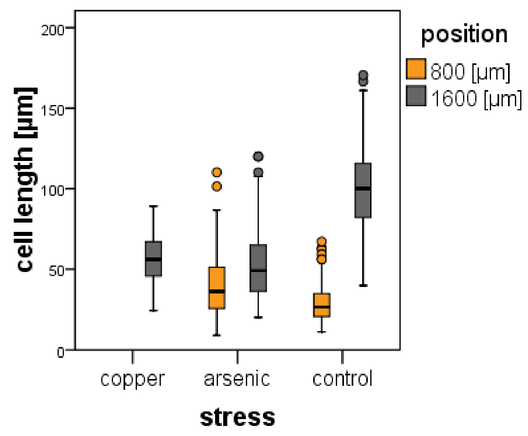

Fig.2: Roots treated with arsenic as well as with copper have significantly shorter root epidermis cells at 1600 µm distance to the root apical meristem. Both treatments cause shorter root epidermis cells.

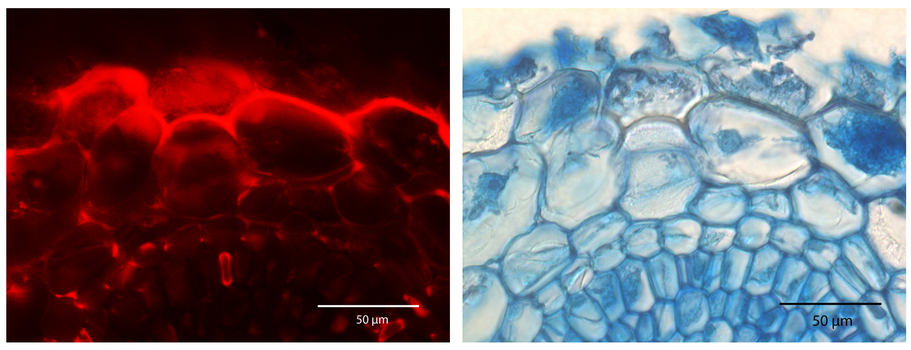

Fig.4: Cross sections of a root treated with copper at 800 µm distance to the root apical meristem. Note the fluorescence of the suberinised outer cortex, the Casparian band and the xylem elements. Root epidermis cells are peeled off.

Results and Discussion

Roots treated with arsenic shift their cell elongation pattern between 0 and 800 µm distance to the root apical meristem (RAM). Furthermore, arsenic causes changes in Ca, Mg, S and Fe content indicating a shift in element translocation and uptake. Although arsenic clearly influences physiology and growth speed, there is no evidence for any change in root anatomy except for the lack of root hairs (Fig.1).

Copper, on the other hand, induces anatomical changes. The root epidermis starts to peel off from the root between 200 µm and 400 µm distance to the RAM. Suberinisation of the outer cortex starts right at the root apical meristem, thus replacing the root epidermis. The Casparian band develops significantly closer to the RAM when compared to controls, indicating that the plant tries to prevent copper uptake. Furthermore, the xylem develops prematurely and changes in the shape of calyptra cells and the whole root can be observed (Fig.4).

Our results give evidence for two hypothesis that may explain the different modes of action of copper and arsenic:

(1) The negatively charged arsenate affects exclusively the cyto-plasm, thus slowing down the growth of the root. The positively charged copper affects the cytoplasm as well, but in addition it influences the mechanical properties of the cell wall and induces changes in the shape of the cells and in the whole root (Fig.3).

(2) Arsenate reduces only root hair formation (Fig.1). The lack of additional barriers results in higher uptake rates of arsenic compared to copper. Copper, on the other hand, triggers defense mechanisms on the anatomical level resulting in the lack of root hairs and in premature suberinisation of cell walls (Fig.4).

References

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