Copper impacts on grape berry cells: uptake and detoxification

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Copper has been extensively used as the active principle of fungicides, since the late 1800s when the "Bordeaux mixture" was developed and its spectacular efficiency proved against fungal pathogens such as downy mildew, which is a large threat to winegrowers. Although initially it seemed to improve plant growth in unproductive lands, repeated use of copper-based fungicides has led to the accumulation of large concentrations of this metal ion in vineyard soils and raised concerns regarding phytotoxicity. As major targets for heavy metal stress, plants have developed a number of mechanisms to withstand the elevated metal levels. Such responses include exclusion, chelation and compartmentation of metal ions. Both the mitochondria and plastids are copper sinks, and the vacuole is believed to constitute a copper delivery pathway within the cell, and not just a sequestration compartment, due to the proximity of the tonoplast to the other organelles of the plant cell. In the present study, grape berry cells (cv. Cabernet Sauvignon) were used as a model system to study the effect of copper on cell growth and viability. In the concentration range of 0 (+ the copper chelator BCS) to 100 µM $CuSO_4$ growth was virtually unaffected. However, concentrations from 100 to 500 µM caused a sharp decrease in cell growth. The viability of grown cells decreased with the increase in copper concentration in a dose-dependent manner. Studies with the copper-sensitive fluorescent probe PhenGreen™ SK allowed for the identification of copper sinks in grape berry cells. Furthermore, transport studies were performed in isolated intact protoplasts loaded with this probe. The initial velocities of fluorescence quenching upon addition of copper followed a Michaelis-Menten kinetics, suggesting the involvement of mediated transport with a K_m= 0.7 mM. Isolated vacuoles labeled with the pH-dependent fluorescent dye ACMA were used to study copper compartmentation as a mean of metal tolerance. Results showed that $CuCl_2$ dissipates a pre-established pH gradient across the tonoplast suggesting the involvement of a Cu^{2+}/H^+ antiport system. Eight putative *VvCTr* (*Vitis vinifera* Copper Transporter) genes were identified, among which *VvCTr1* was isolated and cloned and its expression is currently being studied.

Acknowledgements: VM is supported by a PhD grant (SFRH/BD/64587/2009). This work was supported by Fundação para a Ciência e a Tecnologia (research project no. PTDC/AGRALI/100636/2008).