PRODUCTION OF MYOGLOBIN IN PLANTS FOR BIOTECHNOLOGICAL APPLICATIONS IN THE AGRI-FOOD SECTOR

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Cultivated meat is an innovative, quickly developing technology that is expected to substitute traditional meat production processes, drastically mitigating water footprint, GHGs emission, land use, deforestation and biodiversity loss caused by them (Hubalek *et al*., 2022). However, there are plenty of challenges for this technology to become economically competitive: among these, cultivated meat products lack the conventional red color of meat due to the absence of myoglobin (Fraeye *et al*., 2020).

Heme is an iron-binding tetrapyrrole that is a fundamental component of myoglobin: in fact, the red coloration is provided by the iron atom of the heme group binding to oxygen; moreover, heme-bound iron is much more bioavailable than non-heme-bound iron (Fraeye *et al*., 2020). In plants, heme metabolism is carried out completely in the chloroplast (Richter et al., 2023). There is evidence some of the enzymes involved in heme metabolism have a role in plants salt stress tolerance: heme oxygenase 1 (HO1) - the first enzyme in heme degradation - is known to interact in humans with 14-3-3 proteins (Song et al., 2019), a family of conserved regulatory proteins which are known, in plants, to be involved in several processes such as abiotic stresses tolerance (Huang et al., 2022; Visconti et al., 2019); moreover, it was recently found out that ferrochelatase 1 - the enzyme that directs the tetrapyrroles metabolism towards heme production - is overexpressed under salt stress and it has a role in tolerance mechanisms to it (Zhao et al., 2017).

As of these pieces of knowledge, we propose a plant genetic engineering approach that should yield ovine myoglobin-producing plants where heme levels are increased by knocking out the HO1 gene. The first transformation attempt will be carried out on *Arabidopsis thaliana*, where verification of myoglobin expression, correct heme incorporation and heme titers will be carried out. Then, myoglobin will be expressed in tomato plants - after an *ad hoc* CRISPR-Cas9-mediated HO1 mutation, possibly. Tomato-expressed myoglobin will then be used in ovine muscle satellite cells cultures for 3D meat production, and its role in enhancing its otherwise white color will be evaluated.

Then, the interaction between HO1 and *Arabidopsis* 14-3-3 proteins will be evaluated by interaction assays in 14-3-3 overexpressing lines, already available in our laboratory. Additionally, the role of this interaction, heme, and HO1 in salt stress tolerance will be investigated in wild type and in *ho1* loss of function *Arabidopsis* plants.

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