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Morphogenic responses of Arabidopsis explants cultured *in vitro* in relation to the level of oxidative stress

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Oxidative stress is defined as an imbalance between antioxidants and oxidants activity in favor of the latter. The reactive oxygen species (ROS) are required in low concentrations for normal growth and development while under high ROS level various cellular components (proteins, lipids, carbohydrates, nucleic acids) can be damaged. ROS production is controlled by antioxidants which prevent a cell from excessive damages. The oxidative stress can be generated during natural biological processes in cells (e.g. aging) or it is induced by unfavorable environmental factors (e.g. water scarcity). Oxidative stress factors were indicated to modify embryogenic potential of *in vitro* cultured plant tissues and numerous genes related with stress responses were found among those differentially expressed in embryogenic cultures of different plants. Accordingly, it is believed that somatic embryogenesis (SE) is a specific response of plant somatic cells to oxidative stress related to *in vitro* culture conditions.

In the present study the role of oxidative stress in the mechanism governing SE induction was evaluated in the culture of Arabidopsis explants. In a culture of immature zygotic embryos (IZEs) different morphogenic responses were induced, including somatic embryo (SE), adventitious shoot (ORG) and seedling (E0) development. To modify the oxidative stress level, the culture media were supplemented with glutathione (GSH) and alloxan (AL) at various concentrations. In the presence of GSH (antioxidant) a reduced level of oxidative stress is expected. In contrast, under AL treatment (increased production of ROS) an increased level of oxidative stress in the culture is produced. The effect of these chemicals on the efficiency and productivity of SE was analyzed to evaluate the relation between the level of oxidative stress and the embryogenic potential of the culture. In addition, the impact of different oxidative stress levels on shoot (ORG) and seedling (E0) development was also analyzed.

It was observed that in explants cultured on an auxin medium both, a reduced and an increased level of the oxidative stress impaired the embryogenic response of explants. In contrast to the auxin medium, a positive impact of the reduced oxidative stress level on the embryogenic potential of the culture was observed on a hormone free medium. It was found that a low level of oxidative stress may substitute for auxin treatment required to induce SE in the control culture and somatic embryo formation was observed in explants cultured on a hormone-free medium supplemented with 0.1 mM and 0.5 mM of GSH. Moreover, GSH treated cultures induced towards ORG were found to produce an increased number of shoots per explant.

The results showed that GSH treatment of cultures distinctly affects the morphogenic potential of Arabidopsis explants, including (I) stimulation of shoot regeneration on hormone media (II) reduction of embryogenic response on auxin medium and (III) induction of SE on auxin free medium. In conclusion, the results of the study indicate a significant impact of oxidative stress level on the morphogenic potential of explants cultured *in vitro* and shows that GSH treatment can replace the auxin required for SE induction.