

One of the features that distinguish plants from animals is the fact that the majority of plant cells do not lose their developmental potential and under specific conditions can return to a totipotent embryogenic state. One of such processes called 'microspore embryogenesis' (ME) starts with the reprogramming of immature pollen grains (microspores) and finishes with the formation of so-called 'embryo-like' structures that closely resemble zygotic embryos and can regenerate into haploid (n) plants. Then, after spontaneous or chemically-induced genome doubling so-called doubled haploids (DHs) are produced. As a method for instant totally homozygous line production, ME is highly advantageous in many research areas, bioengineering and plant breeding. However, high genomic dependence and usually low effectiveness limit the implementation of ME on a larger scale in the case of many economically important plants species.

Intensive studies over the past few decades focused on the cellular processes during ME have not succeeded yet in the understanding of the major mechanisms that govern the initiation of embryogenic development of immature pollen grains.

It is well-known that the main factor responsible for the change of microspore developmental pathway is exposure to stress. Surprisingly, various stress factors like high or low temperature, starvation or osmotic stress can act as equivalent triggers for ME induction suggesting a rather unspecific mechanism of its action. It has been hypothesized that such effect can be associated with reactive oxygen species (ROS) generation. These partially reduced or activated derivatives of oxygen are unavoidable by-products of all aerobic processes taking place in a living cell. At low concentrations ROS act as signaling molecules involved in the control and regulation of many physiologically important processes. However, excessive ROS production that usually accompanies any stress treatment can lead to cell death via oxidative destruction. In plant cells, the proper equilibrium between ROS generation and decomposition is critical and precisely controlled by the activity of enzymatic and non-enzymatic components of the antioxidative system. The aim of the project is the verification of this hypothesis by detailed study of oxidative stress modulation and its effect on antioxidative system activity as well as ME efficiency in isolated microspore cultures of two cereal species: triticale (*Triticosecale* Wittm.) and barley (*Hordeum vulgare* L.).

In order to gain a better understanding of the mechanisms that control ME, ROS generation and the activity of enzymatic and non-enzymatic antioxidants will be analyzed in microspores of triticale and barley genotypes differing significantly in respect of ME responsiveness. The intensity of ME-inducing stress will be modified by application of various redox homeostasis-modulating treatments and correlated with ME effectiveness. Then, RNA-sequencing technique will be used for the analysis of genes expression in microspores treated by highly ME-diversifying stress conditions. Final verification of received results will be conducted by the targeted knock-out of candidate gene(s) and the estimation of its effect in relation to ME.