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# Holistic analysis of signaling molecules involved in the gibberellins/ abscisic acid - dependent response mechanism of *Triticum aestivum* seeds to pre-sowing treatment with magnetic field

## Project description

Currently, every year new evidences are revealing the ability of plants to perceive and respond to varying magnetic field (MF) by altering their phenotype and even their gene expression but the details of biological action of MF and its mechanism are still insufficiently understood. Over the years, research results have been also collected to determine the effect of the MF used as a safe alternative method to improve agricultural crops. The most important applications of the MF include treatment of irrigation water, dry seeds, wet seeds and seedlings.

Our preliminary study allowed us to show the high sensitivity of germination of wheat (*Triticum aestivum*) seeds to specific intensity of MF. In the process of cereal seed germination, the key step is to change the balance between the level of two plant hormones, gibberellin (GA) and abscisic acid (ABA). In germinating seeds GA and ABA interact with many intracellular signaling molecules, including reactive oxygen species (ROS), nitric oxide (NO), cyclic nucleotides (cNMP) and calcium ions ($Ca^{2+}$).

During germination of cereal seeds, the developing embryo releases into the cells of the aleurone layer large amounts of GA, which in cooperation with different signaling molecules induce hydrolytic enzymes, necessary to activate storage materials in seeds. In plant cells, GA/ ABA levels interact closely with ROS. It has been proven that ROS affect the reduction of ABA level, causing stimulation of GA synthesis and interruption of seeds dormancy. The activation of proteins in the aleurone layer in cereal seed is in turn regulated by NO together with cyclic GMP. Calcium ions, $Ca^{2+}$, are another signaling molecules crucial in the germination process. In the apical root meristem, the calcium/ kalreticulin protein complex accumulates in large quantities which is associated with the process of cell growth and cell division. Following the release of

$Ca^{2+}$ from the intracellular stores, another protein calmodulin is activated, which increases the activity of catalase enzyme, causing lowering of $H_2O_2$ level in germinating seeds.

Based on the presented facts above, the aims of the proposed doctoral study will be to bring closer to knowledge the involvement of crucial signaling molecules (ROS, NO, cNMP, $Ca^{2+}$) in the process of MF sensing in germinating seeds of wheat (*Triticum aestivum*). A more holistic insight into the role of signaling molecules in the mechanism of gibberellins/ abscisic acid -dependent response under action of two very different MF values, as a pre-sowing treatment, will be given. Moreover, during MF treatment, we plan to tightly control the value of electric field, which is new and lacking approach in scientific experiments concerning MF effects on plant cells. Therefore, the tasks of PhD project will sequentially include:

1. Treatment of dry and wet seeds of wheat with two values of MF: 7 mT (strong MF) and 40 nanoT (below geomagnetic field) with control the GMF value (50 microT).

2. Analysis of hormonal balance of GA/ ABA in MF treated and control seeds:

2.1. Measurement of endogenous GA (selected ones) and ABA levels with chromatography method

2.2. Analysis of expression level of chosen genes of GA biosynthesis and catabolism (*GA20ox*, *GA3ox*, *RGA*)

2.3. Analysis of expression level of chosen genes of ABA biosynthesis and catabolism (*Nced*, *Zep*, *CYP707A*)

3. Analysis of level of crucial signaling molecules in MF treated and control seeds:

3.1. Reactive Oxygen Species (ROS)

3.1.1. Determination of hydrogen peroxide level will by spectrophotometrical method

3.1.2. Localization of ROS in seed tissues with two dye methods - DCFH diacetate for hydrogen peroxide and XTT, WST-1 compounds for the superoxide radical (O2 • -)

3.1.3. Measurements of ascorbate and glutathione levels with spectrophotometric methods

3.1.4. Analysis of activity of 4 antioxidant enzymes: catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) and ascorbate peroxidase (APX) by classical methods biochemical

3.1.5. Analysis of the expression of genes encoding CAT, SOD, POX and APX proteins by real-time PCR (qRT-PCR)

3.2. Nitric Oxide (NO)

3.2.1. Measurements of NO emissions in seeds by chemiluminescent and electrochemical methods

3.3. Cyclic nucleotides (cNMP)

3.3.1. In vitro analysis of cyclic GMP and AMP level by ELISA method and immunocytochemistry

3.4. Calcium ions ($Ca^{2+}$)

3.4.1. Analysis of distribution of freely bound calcium in seeds using transmission electron microscope

3.4.2. Confirmation of the presence of calcium ions in seeds tissues by spectroscopy with EDS energy dispersion

3.4.3. Analysis of the level of two calcium binding proteins: calmodulin (CaM) and calreticulin (CRT) - Western blot with specific anti-CaM antibodies, anti-CRT

3.4.4. Immunolocalization of CaM and CRT proteins in cells of germinating seeds by microscopy

In the project we want to answer the question whether changes in the level of specific signaling molecules and hormones are the basis of the mechanism of response to pre-sowing treatment of wheat seeds with magnetic field. The obtained answer will allow the modification of seed response to MF by the use of specific activators / inhibitors of individual signaling molecules and hormones. This will allow for a more effective use of MF as a physical factor improving germination of wheat seeds. Therefore, our project has also potential for practical application.

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